

Essential Medical Microbiology and Immunology

Vol II

VISIT...

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CONTRIBUTORS

(In alphabetical order)

Abdel-Fattah M. Attia

Azza E. Badr

Fatma A. Mitkees

Hala M. Safouh

Hicham Ezzat

Marie Abdel-Aziz

Mikhael Nasr Mikhael

Mona A. Abdel-Messieh

Mona M. Ezzat

Nagwa A. Sedky

Reda M. Eskander

Sohair A. El-Attar

Sohair A. Elian

Soliman Aref

Somaia A. Eissa

Professors of Medical Microbiology and Immunology

Medical Microbiology and Immunology Department,

Faculty of Medicine, Cairo University

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Genus "Cap letters"
species small "

Chapter 1

مقدمة MICROBIAL TAXONOMY

Taxonomy is the science of biological classification. It consists of three parts: **classification**, **nomenclature**, and **identification**. **Classification** is the arrangement of organisms into groups (taxa). **Nomenclature** refers to the assignment of names to taxonomic groups. **Identification** refers to the determination of the particular taxon to which a particular isolate belongs.

Taxonomic Groups

Organisms (except viruses) are placed in taxonomic groups which include Domain, Kingdom, Phylum, Classes, Orders, Families, Genera, Species and strains in a descending order. Viruses are classified differently, so that the family is the highest taxonomic group.

The basic taxonomic group is the species. Species are defined on the basis of phenotypic and genotypic differences among bacteria.

A **bacterial species** is a collection of strains that share many stable properties and differ significantly from other groups of strains.

A **strain** is an individual member within the species.

A **genus** is a well-defined group of one or more species that is clearly separate from other genera. صنفه ثابتة (نفس الطور مثلا)

The **binomial system** is used for nomenclature of organisms (except viruses). It employs the genus and species names. The genus name is always capitalized (e.g. *Escherichia*) while the species name is never capitalized (e.g. *coli*); both terms are italicized (e.g. *Escherichia coli*) or underlined (e.g. Escherichia coli). After first usage in a manuscript, the first name will often be abbreviated to the first letter (e.g. *E. coli*).

Criteria for Classifying Bacteria

Classification is based on several parameters:

- 1. Genetic homology:** is a similarity between the DNA or the RNA of organisms. It may be determined by base composition, nucleotide sequence or DNA hybridization rates.
- 2. Phenotypic properties:** Properties such as biochemical reactions, chemical composition, cellular structures and immunological features are used in defining a bacterial species.

Accordingly, two organisms are considered to belong to the same species if they have:

1. DNA homology of $\geq 70\%$, or
2. 16S rRNA sequences are $>97\%$ identical.

Systematic Microbiology

Systematic microbiology presented in this book will include a variety of medically important bacteria, fungi and viruses. The systematic study of these microbes will include basic knowledge in order that the student be able to:

1. Describe morphology and structure of infectious microbial agents.
2. Know cultural characteristics of the organism including O_2 requirements, medium for primary isolation and incubation conditions.
3. Know essential biochemical reactions of the microbe.
4. Identify source of the microbe in nature and mode of transmission.
5. Know major disease(s) caused by a particular infectious agent.
6. Recognize key events in pathogenesis, including pathway leading to disease, virulence factors and mechanisms by which microbes cause disease.
7. Specify steps of the laboratory diagnosis of the infectious disease.
8. Know principal antimicrobial agents used to combat infectious agents in human body.
9. Know how microorganisms can be controlled outside the human body.

MCQ
7/10
Q1. 17

* Catalase system used to differentiate
 1. Staph aureus from staph
 2. strept. lyxine from strept pneumoniae
 3. Staph - - - - from strept.
 (A) bec. staph catalase +ve
 & strept catalase -ve

* Bacteria has carrier state in
 7/10
 201
 Staph
 strept agal.
 pneumococcus
 Neisseria meningitidis
 Listeria 10%
 diphtheria
 salmonella
 Shigella

mannitol salt agar
 differential selective
 med.
 for staph
 aureus

Chapter 2

بکتریا العقودیه الکرویه

STAPHYLOCOCCUS

Genus

Gram +ve

Staphylococcus is a large genus, containing at least 30 species which have the following characteristic features:

1. Gram-positive spherical cocci arranged in grape-like clusters.
2. Catalase positive. *MCQ
3. Opaque, pigmented colonies are usually produced on agar. (produce endospores)

① stain
② shape
③ morphology

The ability to produce staphylocoagulase divides the genus into two groups "coagulase-positive" and "coagulase-negative" staphylococci. S. aureus, which is coagulase-positive, has the greatest pathogenic potential and is the most medically important species. The coagulase-negative staphylococci are far less pathogenic. However, S. epidermidis and S. saprophyticus are important coagulase-negative species that can cause infections in man.

Habitats Staphylococci usually inhabit the skin and mucosa. The nose is the main habitat for S. aureus and S. epidermidis. Nasal carriage rate for S. aureus may account to more than 40% in adults.

* Carrier = apparently healthy individual who carry pathogenic organism without show manifest. Can transmit disease (organism) to other individuals.

7 points

Staphylococcus aureus

From 40% Carrier State

Staphylococcus aureus is one of the major pathogens that cause both community-acquired and hospital acquired (nosocomial) infections.

Morphology

S. aureus strains, like other staphylococci, are Gram-positive spherical cocci (about 1 μ m in diameter) occurring in irregular grape-like clusters.

Cultural characters

S. aureus is a facultative anaerobe and usually grows in simple nutrient media. On blood agar, it usually forms large golden yellow colonies surrounded with a zone of β -haemolysis (complete haemolysis) due to production of haemolysins. The organism can tolerate high concentrations of sodium chloride and ferments mannitol. Based on these 2 properties, a selective indicator medium named mannitol salt agar is formulated to facilitate isolation of S. aureus from specimens contaminated by other bacteria.

any organism
7 points

- ① morphology
- ② culture characteristics
- ③ virulent factor
- ④ disease caused by it

تباين الکرولی
9 الکرولی
بسیب ال
hemolysin

It is more blessed to give than to receive.
 * Staph can resist intracellular killing by phagocytosis because it's Catalase +ve

G.R. staph. is localized?
 → @ staphylocoagulase **عكاز**

Virulence factors and pathogenesis

The following virulence factors play important roles in the pathogenesis of *S. aureus* diseases:

most important virulence factors

leucocidin
 staphelastin
 pyolysin

1. **Staphylocoagulase**: Coagulase is an extracellular protein that has the ability to convert plasma fibrinogen to fibrin. By this mechanism, a fibrin barrier is formed. Thus, bacteria could protect themselves from phagocytic and immune defences. At the same time, it leads to localization of infection. Thus *S. aureus* suppurations usually have a localized form, e.g. furuncles.

2. The **clumping factor** (fibrinogen-binding protein) is an important **adhesin** expressed by *S. aureus*. It leads to attachment of the organism to traumatized tissue and blood clots.

3. **Invasins**: Leucocidin, staphylokinase and hyaluronidase promote bacterial spread in tissues. = fibrinolysin

4. **Protein A**: is present on surface of *S. aureus*. It combines non-specifically with Fc-portion of IgG leading to inhibition of opsonization.

5. **Haemolysins** (e.g. alpha toxin): are pore-forming toxins that lyse host cell membranes. They cause haemolysis on blood agar. (Bhaemolysis)

6. **Exotoxins having superantigen mechanism** (see vol. I):

a- **Enterotoxins** responsible for staphylococcal food poisoning.

b- **Toxic shock syndrome toxin-1 (TSST-1)**.

c- **Epidermolytic (exfoliatin) toxins**: responsible for staphylococcal scalded skin syndrome (SSSS).

Staphylococcus aureus Pyogenic Diseases

* **Characteristics**
 1. *S. aureus* infections are frequent, often pyogenic and localized. *S. aureus* exists in a carrier state in the nose of many individuals. It may be found on bed linen and clothing. It is usually transmitted by contaminated hands. Fomite and contact with respiratory secretions also play a role in disease transmission. The pyogenic infections include:

frequent localized Pyogenic

(I) **Pyogenic infect**:

I. **Localized skin infections** are by far the most common:

- a- **Folliculitis**, **furuncles**, **carbuncles**, or **abscesses**.
- b- **Postoperative surgical wound infections** (hospital-acquired).
- c- **Traumatic wound infections** following skin injury and burns.

II. **Staphylococcal pneumonia** is a frequent complication of prior viral infections (e.g. measles, influenza). So in viral ill many antibiotic given to prevent infection

III. **Invasive conditions** are more serious and usually occur in immunocompromised individuals. Invasion of bloodstream (**bacteraemia**) and spread to numerous body sites lead to deep seated infections such as **osteomyelitis**, **endocarditis** and **meningitis**. A resulting **septicaemia** may be rapidly fatal.

Can cause

فوق ٤٠ مليون



Gram + Catalase → staph aureus
Coagulase → S. aureus

Food poisoning → 7
Vd III P. 78

Laboratory diagnosis

- A. Specimens may include pus, sputum, urine, CSF, bloodetc.
- B. Direct detection in clinical specimens by microscopic examination of Gram-stained smear: Gram-positive cocci are seen in clusters in association with pus cells. Microscopy cannot discriminate staphylococcal species.

C. Cultivation

- Specimens other than the blood should be plated directly onto blood agar and mannitol salt agar and incubated at 37°C. BCT
- Blood samples should be cultivated by the blood culture technique in cases of bacteraemia, septicaemia and endocarditis. Subcultures are plated on blood agar and incubated as above.

if sterile sample e.g. CSF which no bact. involved

* D. Identification: After 24h incubation, colonies should be examined for morphology, Gram stain and catalase. Colonies showing Gram-positive cocci in clusters and are catalase positive should be tested for further characters:

- Colony on blood agar is golden yellow in colour and usually surrounded with a clear zone of complete haemolysis.
- Colony on mannitol salt agar is yellow due to change in colour of phenol red indicator as a result of acid production from mannitol.
- Production of staphylocoagulase.
- Presence of the clumping factor.

* yellow on mannitol salt. due to mannitol fermentation (not due to endotox or exotox ??)

Coagulase and clumping factor are the most important markers for identifying S. aureus in the laboratory. They result in aggregation of the S. aureus cells by different mechanisms.

Prevention and control

no vaccine → general hygiene

Improved hygiene and management practices in hospitals are the most effective methods of infection control, especially hand washing and meticulous attention to aseptic techniques related to patient care. Vaccines are still for trials.

Staphylococcus aureus Toxin-Mediated Diseases

enterotoxin
TSST-1
epidermolytic

I. Staphylococcal food poisoning: It is the commonest type of bacterial food poisoning. The disease is caused by at least six antigenic types of enterotoxins (A, B, C, D, E and G) produced by ~50% of S. aureus strains. These toxins use a superantigen mechanism (see vol. I).

= exotoxin heat stable preformed

The source of food contamination is usually a carrier such as foodhandlers harbouring S. aureus on their hands or in the nose, in a boil or a furuncle. The organism grows in the food and produces the toxin.

Heat stable exotoxin → exception for exotoxin

Food poisoning
① Staph. aureus
② Listeria
③ Bacillus cereus

السم لا يتغير بعد اكله او شربه



Staphylococcal enterotoxins do not change the characters of the food regarding its taste, colour or odour. In addition, heating may kill the organism but does not destroy the toxin since it is heat-stable (for ~30 minutes of boiling).

Common types of food include protein-rich food like mayonnaise, milk and its products (e.g. ice cream), or carbohydrate-rich food e.g. cake, pasta and koskosi.

The disease is characterized by very short incubation period of 1-6 hours after consumption of foods containing preformed toxin and manifests as violent vomiting and diarrhoea, but no fever. It is usually self limited. * **No fever**

* **Laboratory diagnosis:** Specimens are usually collected from food remnants, vomitus and/or faeces. These specimens should be tested for the causative S. aureus and/ or its enterotoxin. The specimens are usually contaminated with other bacteria, so selective media such as salt broth and (mannitol salt agar) are used. The isolated strains should be tested for toxin production.

If outbreaks occur, it will be necessary to isolate the toxin-producing strain and trace its source (a nasal carrier or infected skin of a food handler) by performing strain typing (see below).

II. **Toxic shock syndrome (TSS):** TSS is due to infection or colonization by TSST1-producing S. aureus. TSS is prevalent in young menstruating females who use vaginal tampons that are left in place for extended period. Onset of the disease is about 5 days from onset of menses. The syndrome can also occur in men and nonmenstruating females suffering from S. aureus infections anywhere in the body. The disease is characterized by sudden onset of high fever, diarrhoea, vomiting and red rash, plus hypotension with cardiac and renal failure due to the

superantigen action of TSST-1. This results in production of high levels of cytokines that produce the dramatic symptoms. The mortality rate has been 10-15%. * **Diagnosis** usually depends on clinical findings but it can be made by examining vaginal swabs or tampons by culture and/or ELISA that detect TSST-1 in the blood.

III. **Staphylococcal scalded skin syndrome (SSSS):** occurs in neonates and children under 5 years of age. This condition follows infections caused by S. aureus that produces exfoliatin toxins. Large bullae are formed under the epidermis, which rupture leaving moist, red, scalded dermis.

* **Strain typing for epidemiologic investigation (8)**

Typing of strains of S. aureus can be obtained by colony morphology, biotype profiles, phage typing, plasmid analysis, ribotyping, chromosomal analysis and PCR. Strain typing is required in the epidemiologic studies of outbreaks of S. aureus diseases such as food poisoning and hospital acquired infections.

* What can be done if outbreaks recurred?

minor → no antibiotic
severe or abscess
→ antibiotic
surgical
drainage

Treatment and Antibiotic Susceptibilities

Minor skin lesions usually do not need treatment and resolve spontaneously.

Abscesses may require surgical drainage and antibiotic therapy to prevent dissemination. Systemic infections require vigorous antibiotic treatment.

The beta-lactam antibiotics (e.g. penicillins and cephalosporins) are of primary importance for treatment of staphylococcal infections but faced today with emergence of increasing numbers of resistant strains. The following is the most important susceptibility patterns of *S. aureus* isolated from clinical conditions:

1. **Penicillin-resistant *S. aureus*:** Approximately 95% of *S. aureus* strains are resistant to penicillin. This type of resistance is due to β -lactamase (penicillinase) production. Resistance rate is highest among hospital (nosocomial) strains. Resistant strains remain susceptible to the semi-synthetic penicillins (e.g. oxacillin and methicillin), and to cephalosporins.

2. **Methicillin-resistant *S. aureus* (MRSA):** It is a more serious type of resistance. There is a change in the binding site (penicillin-binding protein; PBP) on the organism's cell wall for the drug. Infections caused by MRSA strains cannot be treated with any of the beta-lactam antibiotics including all forms of penicillins. Also, MRSA isolates are often multiresistant to other antibiotics as erythromycin, clindamycin, and tetracycline. Therefore, the choice of antimicrobial agents for treatment should be based on in vitro susceptibility test. However, **vancomycin** can be used empirically as the drug of choice until the susceptibility results are available.

3. **Vancomycin-resistant *S. aureus*:** Unfortunately, during the last few years, some strains of MRSA displayed intermediate (VISA) or full resistance (VRSA) to vancomycin. This situation is very serious since vancomycin is considered the most reliable and effective weapon which is liable to be lost in combating MRSA infections. Since vancomycin is one of the glycopeptides, susceptibility is expressed also as GISA or GRSA. The new antibiotics **linezolid** and **streptogramins** look promising for treatment of infections not responding to vancomycin.

enumerate *

Coagulase Negative Staphylococci

→ *S. epidermidis*

→ *S. saprophyticus*

* ***S. epidermidis*:** is the major cause of infections associated with prosthetic devices and catheters. It has been a pathogen in bacteraemia, native and prosthetic valve endocarditis, infection of surgical wounds, urinary tract, and prosthetic joint.

* ***S. saprophyticus*** is an important opportunistic pathogen in urinary tract infections, especially in young sexually active females.

♀

→ not sexually transmit disease

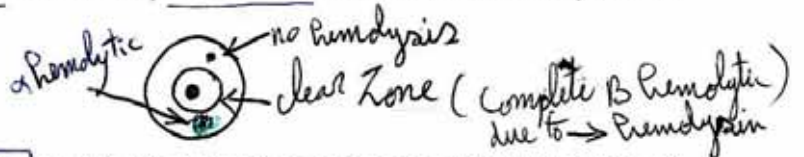
نکته انتقال
من طریق جنسی
نیست

Chapter 3

STREPTOCOCCUS

The genus *Streptococcus* has the following characteristic features:

1. Gram-positive ovoid cocci, arranged in chains or pairs.
 2. Catalase negative: Catalase test is a key test for discriminating streptococci from staphylococci (which are catalase-positive).
 3. Growth requires enriched media containing blood or serum. *not on nutrient media (simple media)*
- Streptococcus pyogenes*, *S. agalactiae* and *S. pneumoniae* are the most important species.



Cultural characteristics

Streptococci are facultative anaerobes and their growth is enhanced in presence of 5% CO₂. Colonies on blood agar are small and do not usually exceed 1 mm in diameter. Haemolysis on sheep blood agar can be used for distinguishing between streptococci as follows:

1. β-haemolytic streptococci induce zones of complete (β-) haemolysis e.g. *S. pyogenes*. β-haemolysis is caused by haemolysins that destroy red cells.
2. α-haemolytic streptococci induce zones of greenish discoloration e.g. *S. pneumoniae*. α-haemolysis is due to release of peroxide that changes haemoglobin to methaemoglobin (or biliverdin). *G.R. α-haemolysis green*
3. Non-haemolytic streptococci are unable to induce any change e.g. *S. bovis*.

Group antigens

These are cell wall carbohydrate antigens called Lancefield group antigens. They are designated A-W (except I and J). Antibodies against these groups are used as one of the tools for identification of *Streptococcus* species.

Classification of *Streptococcus* genus

Haemolytic reactions on blood agar, colony size, Lancefield groups, biochemical reactions, DNA-DNA hybridization, and 16S rRNA sequencing are being used to provide the current classification of the genus *Streptococcus* into 6 groups.

Streptococcus pyogenes (Group A beta-haemolytic streptococcus)

most important
most infection
most diseases
of streptococci

Streptococcus pyogenes represents one of the most impressive human pathogens. It is not only a pyogenic organism but also produces toxins.

What is Lancefield group antigen?

Freely you have received; freely give.

Colonize = Attach
L.T.A. → M-protein
Fib. BP → fibronectin not fibrinogen

Invasion
Antiphagocytic
M-ptn
C5a ppt
Caps. hyaluronic acid

Enz
S.K.
Hyaluronidase
DNase
Staph. L.

Virulence factors and pathogenesis

Streptococcus pyogenes owes its major success as a pathogen to its ability to colonize and rapidly multiply and spread in its host. It produces a wide array of virulence factors:

1. **M protein:** It is one of the cell surface proteins of *S. pyogenes* and represents the most important virulence factor. It enables the bacteria to colonize skin and inhibit phagocytosis. It is immunogenic and divides *S. pyogenes* into about 80 M serotypes.

2. **Fibronectin-binding protein** (Protein F) and **lipoteichoic acids** (LTA) are the main factors that mediate adherence to host epithelial cell surfaces.

3. **C5a peptidase** breaks down C5a so that it no longer attracts phagocytes.

4. **Hyaluronic acid capsule** acts as an immunological mask to avoid phagocytosis. Hyaluronic acid is chemically similar to that of host connective tissue. Therefore, it is not immunogenic. This allows the bacterium to hide its own antigens and to go unrecognized by its host.

5. **Invasins:**

a- **Streptokinase:** It activates plasminogen of human plasma into plasmin that digests fibrin and fibrinogen. Streptokinase (SK) has been referred to as **fibrinolysin** or streptococcal spreading factor. SK is ten times more potent than staphylokinase and is used for emergency therapy of myocardial infarction to remove blood clots.

b- **Hyaluronidase:** It can digest host hyaluronic acid, thus helping spread of infection in tissues.

c- **Nucleases:** They depolymerize DNA. There are 4 DNases (Streptodornases A-D). The B enzyme is the predominant.

d- **Streptolysins:** These are two pore-forming toxins that lyse host cell membranes. They are streptolysin O (oxygen labile) and streptolysin S (oxygen stable). Streptolysin O (SLO) is a highly immunogenic protein and induces specific antibody formation (its detection is the basis for the anti-streptolysin O test). Streptolysin S (SLS) is non-immunogenic. Both toxins are *in vitro* haemolysins. However, the β -haemolysis produced around colonies of *S. pyogenes* on blood agar under aerobic condition is due to SLS since SLO is O₂ labile.

6. **Pyrogenic exotoxins:** Three different streptococcal pyrogenic exotoxins (SPE A, B and C) have been identified. These toxins use a **superantigen** mechanism. In addition, SPE B acts as a protease. SPE A is referred to as **erythrogenic toxin** or scarlet fever toxin because it is responsible for the red rash characteristic of this disease. These toxins cause **toxic shock syndrome**, **septicaemia**, and **necrotizing fasciitis**.

cause red skin rash

Super antigen

Most important

mask & not immunogenic

clinical use to prevent embolism

SLO SLS pore forming cell

SPE A B C

erythrogenic toxin

not identical

هو شبيه وليس نفساً

G.P. hoc. O₂ stable

Streptococcus pyogenes Infections

1. Pharyngitis (sore throat, tonsillitis)

This condition is the commonest infection caused by *S. pyogenes*. Pharyngitis is usually transmitted via respiratory droplets. It is characterized by pain, redness and swelling of posterior pharynx, accompanied by greyish white tonsillar exudate (membrane), and fever.

2. Scarlet fever

Scarlet fever occurs when pharyngitis or any other type of streptococcal infection is caused by an erythrogenic toxin-producing *S. pyogenes*. It is characterized by development of scarlet red rash.

3. Skin and soft tissue infections

Impetigo (pyoderma): It is a local infection of the superficial layers of the skin with blisters and denuded surface covered with crusts.

Cellulitis and erysipelas: Cellulitis is infection of the deep layers of the skin. Erysipelas is a form of cellulitis accompanied by fever and systemic toxicity.

4. Invasive streptococcal infections

a- **Puerperal fever:** This is a life-threatening infection of the endometrium and surrounding structures complicating delivery or abortion. Septicaemia and toxic shock may occur.

b- **Acute endocarditis:** The condition can occur in individuals with normal or damaged heart valves. It is of rapid onset and is highly fatal.

c- **Necrotizing fasciitis** is an invasive disease associated with severe tissue destruction particularly associated with SPE B. The destructive nature of this condition let people to refer to *S. pyogenes* as "flesh-eating bacteria".

d- **Toxic shock syndrome:** This severe form of invasive infection often begins with skin wounds or minor traumas and rapidly deteriorates leading to necrotizing fasciitis or myositis. Shock, renal failure and acute respiratory distress syndrome are complications of the condition.

Laboratory diagnosis

A. Specimens include throat swab, pus, high vaginal swab, CSF, blood...etc.

B. Direct detection in clinical specimens

1- **Gram-stained smear** is useful only when Gram-positive cocci in pairs or chains are detected in specimens normally devoid of flora e.g. CSF.

2- **Detection of group A streptococcal antigen** in throat swab by an agglutination test using group A antibody.

C. Cultivation

- 1- Specimens other than the blood should be plated directly onto blood agar and incubated at 37°C with 5% CO₂.
- 2- Blood samples should be cultivated by the blood culture technique in cases of puerperal sepsis, necrotizing fasciitis and endocarditis. Subcultures are plated on blood agar and incubated as mentioned above.

D. Identification

- 1- After 24h incubation, colonies should be examined for morphology, Gram stain and catalase.
- 2- Colonies showing catalase negative Gram-positive cocci in pairs or chains can be considered *Streptococcus* species for further identification.
- 3- *S. pyogenes* can be identified by:
 - Its colonies are surrounded with wide zone of beta-haemolysis.
 - Its growth is inhibited by bacitracin (i.e. bacitracin-susceptible).
 - Agglutination by group A antibody.

Streptococcus pneumoniae is inhibited by optochin

Post-Streptococcal Sequelae Acute Rheumatic Fever and Acute Glomerulonephritis

Acute rheumatic fever (ARF) and acute glomerulonephritis (AGN) are non-suppurative conditions. Their pathogenesis is based on the hypothesis of an autoimmune mechanism. ARF follows pharyngitis but not skin infection, whereas AGN is preceded by either skin or throat infection. The association of different M serotypes supports the concept of rheumatogenic and nephritogenic strains of *S. pyogenes*. These pathological events begin 1-4 weeks after an acute streptococcal illness (Table 1).

In the case of ARF, antibodies to M protein cross-react with epitopes on heart myosin and sarcolemmal membrane proteins. This might evoke an inflammatory response (complement activation) that damages heart valves. Recurrence of streptococcal pharyngeal infections is common and usually associated with an increased likelihood of rheumatic fever. Repeated recurrences of rheumatic fever may cause valvular damage. Therefore, life-long antibiotic (penicillin) prophylaxis is recommended following a single attack. Examples of rheumatogenic strains include M types 1, 3, 5, 6 and 18. $(1+5 = 6 \times 3 = 18)$

Diagnosis of ARF No single test is pathognomonic. Diagnosis is usually based on the modified Jones criteria. Diagnosis requires an evidence of recent *S. pyogenes* infection together with two of the five major criteria, or one major and two minor criteria (listed below).

bec. no organism may be few remnant

Modified Jones Criteria

200 = normal <<

<500

1. The evidence of recent streptococcal infection:

- 1- A history of acute tonsillitis (or scarlet fever), or the throat swab culture is positive for *S. pyogenes*.
- 2- Elevation of antistreptolysin O (ASO) titre: Anti-streptolysin O (ASO) is the antibody response most often examined to confirm previous *S. pyogenes* infection in a suspected case of ARF. ASO titre of up to 200 units is considered normal. Most acute rheumatic fever cases demonstrate an elevated ASO titre above 200 units (usually >500).

Also, some ARF cases may require additional antibody test(s), namely antistreptokinase (ASK), anti-DNase B or anti-hyaluronidase. The elevation of ASK or anti-DNase B above 80 units or antihyaluronidase titre (AHT) above 128 units is an evidence of recent *S. pyogenes* infection.

2. **Major criteria:** (1) Carditis. (2) Migratory polyarthritis. (3) Erythema annulare. (4) Subcutaneous nodules. (5) Chorea.

3. **Minor criteria:** (1) Elevated erythrocyte sedimentation rate, positive C-reactive protein or increased white cell count. (2) Fever. (3) Prior history of RF.

* **In the case of AGN**, high concentrations of antigen-antibody complexes are formed and deposited on the basement membrane of kidney glomeruli, where they may provoke an inflammatory response that damages the kidney. The predominant nephritogenic serotypes associated with pyoderma or skin infections are M types 2 and 60, while M types 4 and 25 are associated with throat infections. Recurrence is uncommon, and antibiotic prophylaxis following an initial attack is unnecessary.

Diagnosis of AGN: there are elevated antibody titres for anti-DNase B or antihyaluronidase. In glomerulonephritis following pyoderma or skin infection, the ASO titres are generally low. After throat infection (rare), the ASO titres may be higher.

Table (1): Contrasts of acute rheumatic fever and acute glomerulonephritis.

	ARF	AGN
Pathogenesis	<u>Anti-M protein antibodies cross-react with epitopes on heart myosin and sarcolemmal membrane proteins</u>	<u>High concentrations of antigen-antibody complexes are formed and are deposited in the kidney glomeruli</u>
Age	Any, but most commonly between ages of <u>4-30 years old</u>	<u>Children more than adults</u>
<i>S. pyogenes</i> strains	Rheumatogenic	Nephritogenic
Precipitating infection	Pharyngitis (after 2-4 weeks) but not skin infection	Skin or throat infection (after <u>about 10 days</u>)
Recurrence	Common	Uncommon
Prophylaxis with Antibiotics	Essential	Unnecessary
Sequelae	Heart disease	Rarely, chronic renal failure
Most important serological test	ASO	Anti-DNAase or AHT ASO (doubtful)

Free! you have received; freely give.

* 4 Bact. Cause mening. *Strept. agalactiae*
e. coli
Klebsida

Treatment and Prevention



- **Penicillin** is still uniformly effective in treatment of *S. pyogenes* disease. It is important to identify and treat infections in order to prevent sequelae.
- **Long acting penicillin** is used as a chemoprophylactic agent against recurrent *S. pyogenes* infection to prevent repeated rheumatic attacks.
- **Erythromycin** is used as an alternative for penicillin-allergic patients.

* *Streptococcus agalactiae*

(Group B beta-haemolytic streptococci)

+ capsulated → GBS

- *S. agalactiae* is group B beta-haemolytic, having a polysaccharide capsule. = virulence factor
- Group B streptococci (GBS) are important aetiological agents of infections particularly during the first two months of life.
- GBS infections are acquired by infants at the time of birth (25% of pregnant women are vaginal carriers). → neonatal infect.
- **Diseases caused by *S. agalactiae*:**
 - 1- Neonatal sepsis which may manifest as pneumonia, septicaemia, **meningitis** and bone and joint infections. The baby who survives is frequently seriously handicapped.
 - 2- Serious infections in adults, particularly in cancer and diabetic patients.

Prevention

1. Routine screening of *S. agalactiae* in pregnant women.
2. Administration of ampicillin to colonized mothers during delivery (intrapartum) to reduce neonatal sepsis.

Viridans Streptococci

= α hemolysis

These are alpha-haemolytic streptococci which are normal inhabitants of the oral cavity, gastrointestinal and female genital tracts.

Most viridans streptococci do not produce exotoxins or traditional virulence factors. However, they are responsible for nearly 50% of all cases of **subacute bacterial endocarditis (SBE)** because they can adhere to cardiac valves, especially in people with underlying valvular disease. SBE may occur when dental manipulations or trauma to mucosa of upper respiratory tract, e.g. tonsillectomy, lead to bacteraemia. The organism settles on the prosthetic valve or the deformed heart valves e.g. rheumatic or congenital heart.

Viridans streptococci have also a significant role in **dental caries**.

Laboratory diagnosis of SBE: is carried out by the **blood culture technique** and identification of the isolate from the subculture on sheep blood agar.

Treatment and prevention

Viridans streptococci are relatively resistant to **penicillin**. The use of the synergistic combination of **penicillin** and **gentamicin** in life-threatening infections, such as endocarditis, is essential. A single large dose of **ampicillin** or **amoxicillin** should be given to patients with abnormal heart valves prior to dental procedures to prevent endocarditis.

Streptococcus pneumoniae

Pneumococci

Morphology

Gram-positive, lancet-shaped, capsulated, diplococci that may form short chains. The capsule appears as an unstained zone around the organism. The capsule reacts with the specific antibody and can be seen under the microscope to swell. This is the basis of the **quellung test** which is used to identify pneumococci.

Culture: On sheep blood agar, colonies show α -haemolysis.

Virulence factors

The most important virulence factor is the **polysaccharide capsule** which is **antiphagocytic**. It is **haptin**, constituting about 90 serotypes whose antibodies confer type-specific protection against infection.

Pneumococcal diseases

1. **Pneumonia, meningitis and otitis media** are the three major diseases.
2. Sinusitis and conjunctivitis are also common.
3. Endocarditis and septic pericarditis may also occur.

S. pneumoniae is carried in the nasopharynx of about one third of the adults. Infections are transmitted by respiratory droplets. Individuals at risk include the alcoholics, post-splenectomy, immunosuppressed, infants and the elderly.

Laboratory diagnosis

A. Specimens include sputum, CSF, ear or eye discharge and **blood**.

B. Direct detection

- 1- **Gram-stained smear** of **sputum** sample or **CSF** is the most important rapid diagnostic test.
- 2- **Quellung test** (mentioned above).

- 3- Detection of capsular polysaccharide antigen in CSF by means of slide latex agglutination test or ELISA.

C. Cultivation

- 1- Specimens other than the blood should be plated directly on blood agar and incubated at 37°C with 5% CO₂.
- 2- Blood samples should be cultivated by the blood culture technique in cases of bacteraemia accompanying pneumonia, meningitis and endocarditis.

D. Identification

- 1- After 24 h incubation, the colonies should be examined for morphology, Gram stain and one of the tests presented in table (2).
- 2- *S. pneumoniae* colony shows alpha-haemolysis with depression in its centre.

★ Colonies of viridans streptococci are also encountered on blood agar, while examining sputum for *S. pneumoniae*. Confusion occurs due to similarity in microscopic and colony morphology. Discrimination must be done because the viridans group of streptococci does not cause respiratory tract infections but *S. pneumoniae* is the pathogenic one (Table 2).

Why 3 importance to differentiate viridans & pneumo streptococci bec. viridans non pathog = pneumo pathogenic

Table (2): Discrimination between *S. pneumoniae* and viridans streptococci.

Test	<i>S. pneumoniae</i>	Viridans strept.
④ Growth inhibition by optochin <i>green</i>	+	-
③ Solubility of colonies in bile	+	-
② Capsular polysaccharide antigen detection	+	-
① Quellung test to visualize the capsule	+	-
⑤ DNA probe specific to <i>S. pneumoniae</i>	+	-
⑥ Virulence to the mouse	+	-

فای
نکته
نکته
نکته

* Antimicrobial susceptibility

Third generation cephalosporins (e.g. ceftriaxone) are the drugs of choice. Penicillin resistance in pneumococci has been reported. Combination of vancomycin and ceftriaxone should be used to treat serious infections, like meningitis.

* drug of choice

* Prevention

Vaccine

A capsular polysaccharide vaccine is available which contains antigens from the most common 23 pneumococcal serotypes. The vaccine is used after splenectomy in elderly and immunosuppressed patients.

A new pneumococcal conjugate vaccine is now available. This conjugate vaccine is capsular polysaccharide complexed with a protein carrier that makes the immunogen more effective in inducing a response in infants. It is routinely recommended for all children less than 2 years of age.

meningitis

* Give acc. of ptn con vaccine & examples

① meningococci
② pneumococci
③ Haemophilus
Thymus
S. pneumoniae
vaccine
not given to children < 24

Chapter 4

faecalis
faecium

ENTEROCOCCUS

Intestine → Bile salts

Catalase -ve
Gram +ve
Cocci
= singly in pairs
& short chains

Characters of the genus *Enterococcus*

1. Enterococci are Gram-positive cocci that occur singly, in pairs, and in short chains.
2. They are facultative anaerobes and are able to grow at 45°C. → but
3. They grow in broth containing 6.5% NaCl
4. They hydrolyze esculin in the presence of bile salts.
- * 5. They are catalase negative.
- * 6. Most strains react with the streptococcal Lancefield group D antiserum.

These organisms are found normally in the human intestine and female genital tract. The common 2 species are *Enterococcus faecalis* and *Enterococcus faecium*.

Infections caused by enterococci

1. Urinary tract infections are the most common.
2. Intra-abdominal or pelvic wound infections.
3. Bacteraemia.
4. Endocarditis.
5. Abscesses, meningitis, peritonitis, osteomyelitis, and wound infection.

Enterococcus faecalis is the species most commonly isolated from these infections and is predominantly nosocomial rather than community-acquired.

Antimicrobial susceptibility

Enterococci are frequently resistant to antibiotics.

They are absolutely (intrinsically) resistant to cephalosporins and clindamycin. A synergic combination of a cell wall-active drug (penicillin, ampicillin, or vancomycin) plus an aminoglycoside (gentamicin or streptomycin) should be used for serious infections like bacteraemia, endocarditis or meningitis. Susceptibility testing should be done to select the appropriate combination for synergy.

bcc doesn't have receptors for cephalosporins

G.R.

* Compare strept & enterococcus
Lancefield group

enterococcus الفرق
Hydrolyze esculin in bile salt

Chapter 5

NEISSERIA

فد باله مينايش
Catalase
Coagulase

Gram -ve Cocci (pairs
Kidney shape)
Aerobic
Oxidase +ve (Key test)
sugar fermentation acid only

Characters of the genus *Neisseria*

1. Morphology: Gram-negative cocci arranged in pairs with the adjacent sides flattened to give the characteristic kidney or coffee bean shape. 3
2. Aerobic.
3. Oxidase-positive. Oxidase is a key test for identification of the genus *Neisseria*.
4. Sugar fermentation identifies the species.

The genus includes 2 important human pathogens:

1. *Neisseria gonorrhoeae* (gonococci) causes the sexually transmitted disease gonorrhoea, which is a common venereal disease.
2. *Neisseria meningitidis* (meningococci) causes epidemic cerebrospinal meningitis and meningococcaemia.

Glu fermenter

Neisseria gonorrhoeae

isolated by selective media
e.g. MTM

Morphology (mentioned above)

Cultural characters

N. gonorrhoeae is the most fastidious neisseria species. It requires an enriched medium like chocolate agar but it does not grow on blood agar. Selective media like modified Thayer-Martin medium (MTM) allow easier isolation of the organism from specimens contaminated by other microbes. Cultures should be incubated in a humid atmosphere with 5-10 % CO₂ at 37°C.

Biochemical reactions

N. gonorrhoeae ferments glucose only with production of acid only. It is oxidase positive.

Virulence factors

1. Pili mediate attachment to epithelial cells.
2. Outer membrane proteins for tighter attachment and invasion.
3. Outer membrane porin prevents fusion of phagosomes and lysosomes.
4. Lipooligosaccharides (LOS), a modified endotoxin that elicits an inflammatory response. (not LPS like meningitis)
5. IgA1 protease inactivates secretory IgA1 leading to more adherence to and colonization of the mucosa.

Endotoxin LOS

No capsule here but meningitidis has capsule

GR. gonococci resist intracellular killing?

* Why N. Gonorrhoea persistent?

No gonorrhoea in vagina because high acidity

Diseases Caused by N. gonorrhoeae

I. Gonorrhoea

A. Men: Gonococci infect the urethra leading to acute urethritis with dysuria and purulent discharge. The organism may spread to the prostate, bladder and epididymis causing inflammation and swelling.

B. Women: Gonococci infect the cervix (not vagina), urethra, vulva and rectum leading to dysuria and cervicitis with a purulent cervical discharge. About half of infections in women, however, are asymptomatic and may contribute to persistence and spread of gonorrhoea.

Asymptomatic

Pathogenesis of gonorrhoea: Gonococci are introduced onto mucosal surface by sexual contact. Gonococci cannot survive dryness, so intimate contact is necessary for transmission. Pili and IgA1 protease allow adherence and colonization to mucosa. Endocytosis and intracellular growth cause destruction of epithelial cells. Gonococci are able to survive inside polymorphonuclear leucocytes due to inhibition of fusion of phagosomes with lysosomes.

G.R. transmit sexually bec. need intimate

ophthalmia neonatorum

II. Neonatal conjunctivitis: It is an acute conjunctivitis in infants born to mothers with gonorrhoea. The eyes become infected at the time of delivery, and if untreated can lead to blindness.

III. Vulvovaginitis: Infection of the vagina and vulva in young girls due to sexual abuse.

IV. Pelvic inflammatory disease (PID): It occurs in females due to ascending infection and manifests as endometritis and salpingitis which may lead to ectopic pregnancy or sterility. PID may also lead to pelvic peritonitis, tuboovarian abscess or gonococcaemia.

V. Disseminated gonococcal infection (DGI) or gonococcaemia: It occurs more in females (especially the pregnant) and individuals with defect of the terminal complement components. It may manifest as arthritis accompanied with skin rash, meningitis or endocarditis. DGI may result in disseminated intravascular coagulation (DIC) and shock due to the LOS endotoxin.

Laboratory diagnosis of adult gonorrhoea

Acute gonorrhoea = direct smear only enough urethral discharge
other diseases

A. Specimens: urethral discharge from men and cervical and urethral discharge, and rectal swab from women.

B. Direct detection

kidney shape

1- Gram-stained smears show intracellular, Gram-negative diplococci in some polymorphonuclear leucocytes. Smears of urethral discharge from men with symptomatic gonococcal urethritis are highly sensitive and sufficient for diagnosis of male acute gonorrhoea. A Gram-stained smear of female's specimens or in male chronic cases is less sensitive since the small number

170

of gonococci may be overlooked by the predominant microbial flora.
Cultivation is necessary to reach a diagnosis.

- 2- Direct detection of gonococcal antigen in specimens by ELISA.
- 3- A nucleic acid probe test detects gonococcal nucleic acid in specimens.

C. Cultivation: The specimens are plated onto chocolate agar and MTM.
Cultures should be incubated in a humid atmosphere with 5-10% CO₂ at 37°C. = special requirement for growth

D. Identification

- 1- After incubation for 1-3 days, colonies should be examined by Gram stain -ve and oxidase test. Colonies showing Gram-negative diplococci and oxidase positivity are considered Neisseria.
- 2- The organism is identified as N. gonorrhoeae by:
 - * a. Glucose fermentation with production of acid.
 - b. Detection of specific antigens by agglutination or immunofluorescence.
 - c. Nucleic acid probe.

E. Serology: Detection of gonococcal antibodies in patients' sera is not useful.

N.B.: For diagnosis of other gonococcal infections, the same methods are followed using appropriate specimens e.g. conjunctival swab, blood and joint fluid.

Antimicrobial susceptibility *GRs why give tetracycline in addition to primary therapy even it's resistant to it? bec. it's usually concurrent with Chlamydia infection*
Penicillins and tetracycline are no longer used for treatment due to development of resistance. Third generation cephalosporins (e.g. ceftriaxone) or fluoroquinolones (e.g. ciprofloxacin), or spectinomycin is now recommended for primary therapy of gonorrhoea. Although N. gonorrhoeae is frequently resistant to tetracycline, this agent or azithromycin should be given in addition to those for primary therapy, due to the concurrent Chlamydia infection.

Prevention

*No vaccine * Why recurrent infect.? ...*
Repeated gonococcal infections may occur although gonococcal antibodies are produced. This is due to: a) the high antigenic variations of gonococci, b) the superficial nature of the infection, and c) the production of IgA1 protease.³

Chemoprophylaxis is essential to prevent gonococcal neonatal conjunctivitis. Eye ointment of erythromycin is effective for the newborn.

Neisseria meningitidis

→ Glu + Malt. ferment

N. meningitidis is the aetiological agent of meningococcaemia and meningitis.

Morphology (mentioned previously)

*Gram -ve / oxidase +ve
diplococci
kidney shape*

Cultural characters

N. meningitidis grows well on chocolate agar or blood agar. Modified Thayer-Martin medium (MTM) is required to isolate the organism from contaminated specimens (e.g. nasopharynx). Cultures should be incubated in a humid atmosphere with 5-10% CO₂ at 37°C.

Less fastidious bec. can grow on both blood + chocolate

Gonococcus only
Gla fermenter



Biochemical reactions

N. meningitidis ferments glucose and maltose with production of acid only. As all neisseriae, it is oxidase positive.

Antigenic structure and classification

1. Capsular polysaccharide antigens: These can identify 13 serogroups.
2. Outer membrane proteins: which classify each serogroup into serotypes.
3. Cell wall Lipopolysaccharides (LPS): also classify the organism into serotypes.

Virulence factors

1. The polysaccharide capsule, with its antiphagocytic action, represents the most important virulence factor.
Capsule → Pili → auto memb → memb. protein
2. LPS is responsible for the endotoxin effect of meningococcal infections.
3. Pili are responsible for attachment and adherence.
4. IgA1 protease inactivates secretory IgA1. → *more adherence*

Meningitis and Meningococcaemia

** droplet transmission*

20% of meningitis

Disease and serogroups association

N. meningitidis is the aetiological agent of meningococcal disease, most commonly meningococcal bacteraemia (meningococcaemia) and meningitis. The organism causes 20% of all cases of meningitis which may occur sporadically or in epidemic form (epidemic cerebrospinal meningitis). The most common serogroups causing disease are A, B, C, Y, and W135. Serogroup A accounts for most meningococcal disease cases in Africa and some parts of Asia.

Pathogenesis

N. meningitidis is normally carried in the nasopharynx in 5-30% of healthy population. This carrier state increases immediately before and during epidemics. These carriers represent the source of infection from whom the infection is transmitted by droplets.

- Pili allow the attachment of the organism to the mucosal epithelium of the nasopharynx and together with IgA1 protease establish bacterial adherence.
- Endocytosis takes place and a slight local inflammation (sore throat) occurs.
- ④ The organism enters the bloodstream. From the blood, the organism may settle in different parts of the body. Localization of organisms in the meninges leads to meningitis and cerebral oedema.
- ⑥ The virulence of meningococci is primarily due to the invasive capacity of the capsulated organism.

⑦ The disease manifests as sudden severe headache, projectile vomiting and stiff neck, may progress rapidly to coma and death or resolve with permanent neurological complications e.g. deafness, speech disability, paralysis...etc.

- N. meningitidis may localize also in the joints or endocardium leading to arthritis or endocarditis. Skin rash usually occurs with meningococcaemia due to the endotoxin action of the LPS. In fulminant infections, DIC occurs which leads to shock and may end in death. GR. children^s more liable to infection?
- Particularly susceptible hosts include: a) children under the age of 3 years because they fail to make antibody against the antiphagocytic capsule (TI antigen), and b) individuals having defect in the terminal complement components.

Laboratory diagnosis

Meningitis is a medical emergency, so diagnosis must be rapid and precise.

A. Specimens: CSF, blood and aspirates from joint fluid.

B. Direct detection

- 1- Gram-stained smears prepared from CSF show Gram-negative diplococci, intracellular, in some polymorphonuclear leucocytes.
- 2- Direct detection of meningococcal antigen in body fluids by latex agglutination or fluorescent antibody test.

The result is essential to direct the antimicrobial therapy and must be reported within one hour to the treating physician.

C. Cultivation

- 1- Specimens other than the blood are plated onto chocolate agar, blood agar and MTM. Cultures should be incubated in a humid atmosphere with 5-10% CO₂ at 37°C.
- 2- Blood samples should be cultivated by the blood culture technique. Subcultures are plated on chocolate or blood agar and incubated as mentioned above.

D. Identification

- 1- After incubation for 1-3 days, colonies should be examined by Gram stain and oxidase test. Colonies showing Gram-negative diplococci and oxidase positivity are considered Neisseria.
- 2- The organism is identified as N. meningitidis by:
 - a. Fermentation of glucose and maltose with production of acid only.
 - b. Detection of specific antigens by agglutination or immunofluorescence.
 - c. Nucleic acid probe.

2 sugars fermenter

Treatment and antimicrobial susceptibility

- Third-generation cephalosporins such as ceftriaxone or cefotaxime are currently the drugs of choice for empiric therapy.
- Sulphonamides are no longer recommended due to emergence of resistance.
- Penicillin G has remained effective, but resistance has been reported.

→ Third generation Cephalosporins
of choice
→ Penicillin G → resistant reported

* Blood sample
Can't direct
cultivate on Blood agar bec. solid media

→ Blood culture technique

* Blood agar

- Chloramphenicol is still recommended as an alternative for treatment in penicillin allergic patients.

• Penicillin plus chloramphenicol combination MUST NOT be used due to the antagonistic effect.

Meningitis prevention

A- Vaccination

- Currently, polyvalent vaccines are available that contain capsular polysaccharide from serogroups A and C (bivalent) or A, C, W-135 and Y (quadrivalent, WYAC).

- Group B capsular substance is not yet included in vaccine because it is poorly immunogenic because of its similarity to sialic acid found in human tissues.

- Protein conjugate vaccines are being developed that should be more immunogenic than the bacterial antigens alone especially in children less than two years old who respond poorly to polysaccharide antigens.

- Induced immunity lasts for about 3-5 years. The vaccine provides 80-90% protection to adults but it is generally less effective in young children.

- Vaccination is particularly important to groups at risk e.g. military recruits, school children, and college students as well as travelers (e.g. pilgrims) to certain parts of the world.

B- Chemoprophylaxis is recommended for close contacts, e.g. healthcare and laboratory workers and household contacts and in outbreaks. The antibiotic recommended is either rifampin for 2 days orally or one ceftriaxone injection.

C- Routine use of masks is recommended for the close contacts.

Other Neisseria Species and Related Organisms

- ① ***Neisseria kochii*** has been isolated in Egypt from patients with conjunctivitis and urethritis. *N. kochii* strains have colonial morphology characteristics of *N. meningitidis* and biochemical characteristics of *N. gonorrhoeae*.

- ② **Commensal neisseria species:** These are inhabitants of oro- and nasopharynx of healthy individuals. The commensal neisseriae, e.g. *N. lactamica* and *N. sicca* rarely cause diseases such as endocarditis and meningitis.

- ③ ***Moraxella (Branhamella) catarrhalis*:** It may occur as normal flora of the nasopharynx in humans. It causes respiratory tract infections including bronchitis, pneumonia, otitis media and sinusitis. It may cause endocarditis and meningitis in the elderly patients. *M. catarrhalis* is similar in morphology to neisseriae, but differs in the biochemical reactions.

Aerobic non spore forming Gram +ve Bacilli

Freely you have received, freely give.
remember no need selectivity in sterile sites @ urine @ CSF
③ Blood
needed only if specimen is not sterile e.g. skin stool sputum

* Gram +ve bacilli non sporing
* pleomorphic (dist + ple)
* club shaped

Chapter 6

CORYNEBACTERIUM

Members of the genus *Corynebacterium* are pleomorphic, Gram-positive non-spore forming bacilli. The bacilli show a characteristic club-shape, hence the genus name (club = Coryne). The genus comprises many species; some are part of the normal flora of skin and mucous membranes. The best known and most medically important species is *Corynebacterium diphtheriae*, the causative agent of diphtheria. The term "diphtheroids" is used to refer to other corynebacteria because they resemble *C. diphtheriae* in morphology.

by droplet case

droplet carrier

Corynebacterium diphtheriae

Morphology

C. diphtheriae is club-shaped pleomorphic Gram-positive bacilli arranged at acute angles or parallel to each other, resembling Chinese letters. Metachromatic granules are present and give the bacilli a characteristic beaded appearance when stained by methylene blue stain.

Cultural characters

C. diphtheriae is aerobic and can grow on blood agar and Loeffler's serum media. Growth on Loeffler's serum shows the best morphology. The selective differential media containing tellurite (e.g. blood tellurite agar) are used to isolate *C. diphtheriae* from the pharynx, nose and skin where bacterial flora predominates.

Virulence factors

The virulence factor of *C. diphtheriae* is exotoxin. The ability of *C. diphtheriae* to produce the diphtheria exotoxin is dependent on two elements: (1) the presence of a lysogenic prophage in the bacterial chromosome and (2) low extracellular concentrations of iron. The gene for toxin production occurs on the prophage, but a bacterial repressor protein controls the expression of this gene. The repressor is activated by iron.

The toxin produced by the three biotypes of *C. diphtheriae* (gravis, intermedius and mitis) is antigenically the same. The differences in virulence between them can be explained by the amount of toxin produced (being highest in gravis).

Mode of action: The toxin is composed of A and B subunits. The toxin binds by its B fragment to a specific receptor on susceptible cells. The A fragment inhibits protein synthesis leading to death of the cell. The cardiac muscle and 9th cranial nerve are very sensitive to diphtheria toxin.

local pseudomembrane

② Toxaemia

Pathogenesis

Diphtheria is described as an upper respiratory tract illness transmitted by droplets, from a case or a carrier. The incubation period of diphtheria is 2-5 days. Diphtheria involves both local and systemic pathology:

1. **Local pseudomembrane:** It develops in the upper respiratory tract (tonsil, pharynx, larynx, and/or nose). It forms due to necrosis of the epithelial cells followed by blood leak resulting in a network of fibrin, WBCs and RBCs interlaced with *C. diphtheriae* cells. Thus, an adherent membrane forms and may spread locally and can cause suffocation.
2. **Toxaemia:** The diphtheria bacilli do not invade tissues. They produce the toxin that is absorbed and disseminated through the blood to the susceptible tissues (mainly heart muscle and peripheral nerves). The diphtheria toxin causes death of host cells and tissues.

* No septicemia? no bacteremia + no pyemia * bec. no bacteria in blood

Clinical manifestations

The disease is acute, has an insidious onset and the commonest form is the pharyngeal (or tonsillar) diphtheria. Cervical lymphadenitis ("bull neck") is one of the initial symptoms. It is usually associated with severe toxaemia. Extensive membrane formation may result in respiratory obstruction. The patient appears quite toxic with low-grade fever.

The most frequent complications of diphtheria are myocarditis and neuritis. Death may occur within 6-10 days from heart failure, airway obstruction, or diaphragmatic paralysis.

Laboratory diagnosis

The diagnosis of diphtheria is primarily clinical in order to promptly initiate antitoxin therapy without waiting laboratory diagnosis. Microbiological diagnosis serves to confirm the clinical diagnosis and is of epidemiological significance. Microbiological diagnosis should include isolation of *C. diphtheriae* and detection of its toxigenicity.

A. **Specimens:** Swabs taken from under the pseudomembrane.

B. **Direct detection in clinical specimens**

1- Gram stain shows club-shaped Gram-positive bacilli.

② Methylene blue staining shows the characteristic beaded appearance.

C. **Cultivation:** A variety of media may be used as mentioned above. A blood agar plate is also used for detection of *Strept. pyogenes* for differential diagnosis. → most common in pharyngitis strept pyogenes so included

D. **Identification:** *Corynebacterium* colonies are gray/black on tellurite agar. *C. diphtheriae* may be identified as mitis, intermedius, or gravis biotype by differences in both colony morphology and biochemical tests.

lysozyme + tellurite

↓ identification

” اللهم اني اطلب العلم فيك وكنه انك تقصه على من يشاء ”

E. Toxicogenicity testing of the isolated *C. diphtheriae* strain:

- 1- Elek test: This is the most common *in vitro* assay for toxicogenicity. The production of diphtheria toxin can be detected by the formation of toxin-antitoxin precipitation bands in the agar.
- 2- Tissue culture cytotoxicity tests.
- 3- Detection of toxin gene by PCR.
- 4- Detection of toxin from culture by ELISA.

toxin antitoxin ppt band in agar
Elek in vitro test

Direct → because indirect = detect antibody
→ bec. no pseudomembrane to take specimen

For diagnosis of carriers, throat or nasal swabs are subjected to the same procedures except the direct detection methods.

Treatment

① Cause ② symptoms ③ Complications

start after clinical suspect

A. Diphtheria antitoxin: It must be given immediately if diphtheria is clinically suspected, to limit disease progression. Antitoxin will not neutralize toxin that is already fixed to tissues, but will neutralize the circulating (unbound) toxin. Diphtheria antitoxin is usually produced in horses. The antitoxin may be given intramuscularly or intravenously, taking precautions to avoid hypersensitivity reactions (anaphylactic shock or serum sickness). The patient must be tested for sensitivity before antitoxin is given.

antihistamin + Cortisone + adrenalin

B. Antibiotics: In addition to the diphtheria antitoxin, patients with suspected diphtheria should be given antibiotics in adequate dosage. Penicillin or erythromycin is the drug of choice.

C. Respiratory support and airway maintenance may be needed.

Immunity

Immunity to diphtheria involves an antibody (antitoxin) response to diphtheria toxin following clinical disease or immunization with diphtheria toxoid.

Prevention and Control

Active immunization:

Vaccine not alone → D + tetanus
or → D + pertussis + tetanus

- Diphtheria is a preventable disease by routine active immunization with diphtheria toxoid.

Diphtheria toxoid is prepared by growing toxigenic *C. diphtheriae* in a liquid medium. The filtrate is incubated with formaldehyde to convert toxin to toxoid. It is adsorbed onto an aluminum salt which acts as an adjuvant.

- Diphtheria toxoid alone is not available. It is available either combined with tetanus toxoid (as pediatric DT or adult Td), or with both tetanus toxoid and pertussis vaccine (DPT).

- Vaccine is given by intramuscular injection at 2, 4, 6, and 18 months of age with a booster dose at school entry (6 years). A booster dose of Td is then recommended every 10 years.

Vaccines: ① strept pneumoni
② meningococci
③ diphtheria

Carriers state →

Make Diphtheria



For close contacts, especially household contacts, a booster of diphtheria toxoid should be given. Contacts should also receive antibiotics (benzathine penicillin or erythromycin).

N.B.: Diphtheria antitoxin is no longer indicated for prophylaxis of contacts of diphtheria cases.

Why cause food poisoning? *Listeria* (4 words) *E. Tetani*, *E. Botulinum* (Zoonotic) motile *Listeria* is motile at 28°C. food poisoning 10% Intest. carriers neonatal meningitis

Listeria resembles corynebacteria in morphology but it is motile at 28°C. *Listeria* spp. are isolated from a diversity of environmental sources including soil, water, foods and faeces of humans and animals.

Listeria monocytogenes is the most important species. About 10% of humans may be intestinal carriers of *L. monocytogenes* and the most serious infection is neonatal meningitis. The organism resists freezing, drying, and heat although it does not form spores. This enables *Listeria* to survive food processing and so, *Listeria* has been implicated in several outbreaks of food poisoning.

Gardnerella vaginalis (4 words) due to lactobacilli Gram variable

Gardnerella vaginalis is a Gram-variable, pleomorphic, nonmotile bacillus. It can be found in anorectal flora of healthy adults of both sexes. It is also part of the vaginal flora in women during the reproductive age.

Gardnerella vaginalis could be associated with the following disease conditions:

1. **Bacterial vaginosis (BV)**: It is the most common condition, a nonspecific vaginitis that is accompanied by thin vaginal discharge with a bad odour.
2. Preterm birth, premature rupture of membranes and chorioamnionitis. *
3. Postpartum or postabortal fever.
4. Neonatal infections.

N.B. Bacterial vaginosis + N. gonorrhoea direct swab enough

Laboratory Diagnosis Bacterial vaginosis is diagnosed by making a direct Gram stained smear from vaginal discharge. Predominance of *G. vaginalis*, presence of clue cells (vaginal epithelial cells covered with *G. vaginalis* bacilli), and almost absence of lactobacilli, indicate BV. Culture is usually needed for diagnosis of extravaginal, systemic and neonatal infections.

Erysipelothrix

Erysipelothrix rhusiopathiae is the species of medical importance. It is found in wild and domestic animals, birds and fish. The organism enters through skin abrasions usually in hands of meat and fish handlers. The organism causes erysipeloid (a skin infection that resembles erysipelas caused by streptococci).

Genetics → *Bacillus* (aerobic facultative anaerobic)
 → *Clostridium* (anaerobic)
 Chapter 7

SPORE-FORMING GRAM-POSITIVE BACILLI

The Gram-positive rod-shaped bacteria that form endo-spores, have two principal subdivisions:

- The aerobic or facultative anaerobic genus *Bacillus*.
- The anaerobic genus *Clostridium*.

③ Morphology

Bacillus

Bacillus anthracis
 Gram +ve • large rectangular
 spore forming non motile
 encapsulated & arranged in chains

Bacillus species are found everywhere in nature. Important pathogenic species of humans and animals are *Bacillus anthracis* and *Bacillus cereus*. *B. anthracis* is the causative agent of anthrax. However, the majority of species are harmless saprophytes and generally called **anthracoids**.

Bacillus anthracis

Morphology

Bacillus anthracis is a very large, rectangular, Gram-positive, spore-forming rod. It is nonmotile, encapsulated and arranged in chains. Capsule formation occurs *in vivo*. The capsular material is detected in smears from infected tissues after staining by polychrome methylene blue. Blue rods in a background of purple/pink-stained capsular material are a positive test (**McFadyean reaction**). The bacterium forms oval central non-bulging spores. Spores are formed *in vitro* and only in the presence of oxygen. The spores are stained with a special acid-fast (spore) stain. *B. anthracis* spores may survive in dry soil for up to 40 years.

→ Capsule *in vivo*
 → poly chrome methylene blue (McFadyean reaction)
 → spores with special acid-fast

* survive in dry 40 years

Cultural characters

Bacillus anthracis can be cultivated in simple nutrient media under aerobic or anaerobic conditions. *B. anthracis* grows well on blood agar, forming non haemolytic colonies.

→ simple nutrient media
 → blood agar → non haemolytic colonies
 facultative anaerob

Anthrax

Pathogenesis & virulence

The pathogenicity of *B. anthracis* depends on two virulence factors: the capsule and the exotoxin.

- ① **Capsule:** *B. anthracis* produces a **polypeptide capsule** that protects it from phagocytosis. The capsule of *B. anthracis* has a single antigenic type. It plays its most important role during the establishment of the infection, and a less significant role in the terminal phases of the disease, which are mediated by the anthrax toxin.

→ Main virulence stage 1st 2nd 3rd
 → Capsule early
 → exotoxin late

Sialic acid bec... 27... * no vaccine against capsule because it's poorly

exotoxin

different types ABC
3 types
strep. prod. diphteria (AB)
نوعان

2 Anthrax toxin is analogous to the A-B structure-function model of cholera toxin. The toxin consists of three distinct protein factors:

1. The oedema factor (EF). } (A) domain
2. The lethal factor (LF). }
3. The protective antigen (PA). → (B) domain

EF and LF form the active (A) domain while PA forms the binding (B) domain.

Protective immunity against anthrax requires antibodies against the components of anthrax toxin, primarily the protective antigen. The capsule of *B. anthracis* is poorly immunogenic. because resembles sialic acid in human tissue

Clinical manifestations

Anthrax is primarily a disease of farm animals. Humans acquire anthrax as a result of contact with infected animals or animal products. There is no person-to-person transmission of anthrax. The disease takes one of three forms, depending on the route of infection:

1. **Cutaneous anthrax (malignant pustule = الجمرة الخبيثة)** which occurs from handling infected material. Spores from the soil or an infected or dead animal enter through a cut or abrasion, usually on an exposed area. The spores germinate and vegetative cells multiply locally forming a small papule which changes rapidly to a vesicle, then a pustule and finally into a necrotic ulcer which blackens to form the characteristic eschar. The lesion is painless and is surrounded by marked oedema. Untreated cases may develop fatal fulminating septicaemia.

2. **Intestinal anthrax** from eating infected meat.

3. **Pulmonary anthrax** from inhaling spore-laden dust. The possibility of creating aerosols containing anthrax spores has made *B. anthracis* a chosen weapon of bioterrorism. Why? → bec. easily creat aerosol contain anthrax spore

Diagnosis

The clinical diagnosis of anthrax is confirmed by directly visualizing or culturing the anthrax bacilli. Demonstration of capsulated *B. anthracis* from human and animal specimens, even in low numbers, confirms a clinical suspicion of anthrax.

Bacillus cereus

Bacillus cereus causes two types of food poisoning:

1. The "short-incubation" or **emetic form**: It has an incubation period of 1 to 6 hours and is characterized by nausea, vomiting and abdominal cramps. It resembles *Staphylococcus aureus* food poisoning in its symptoms and

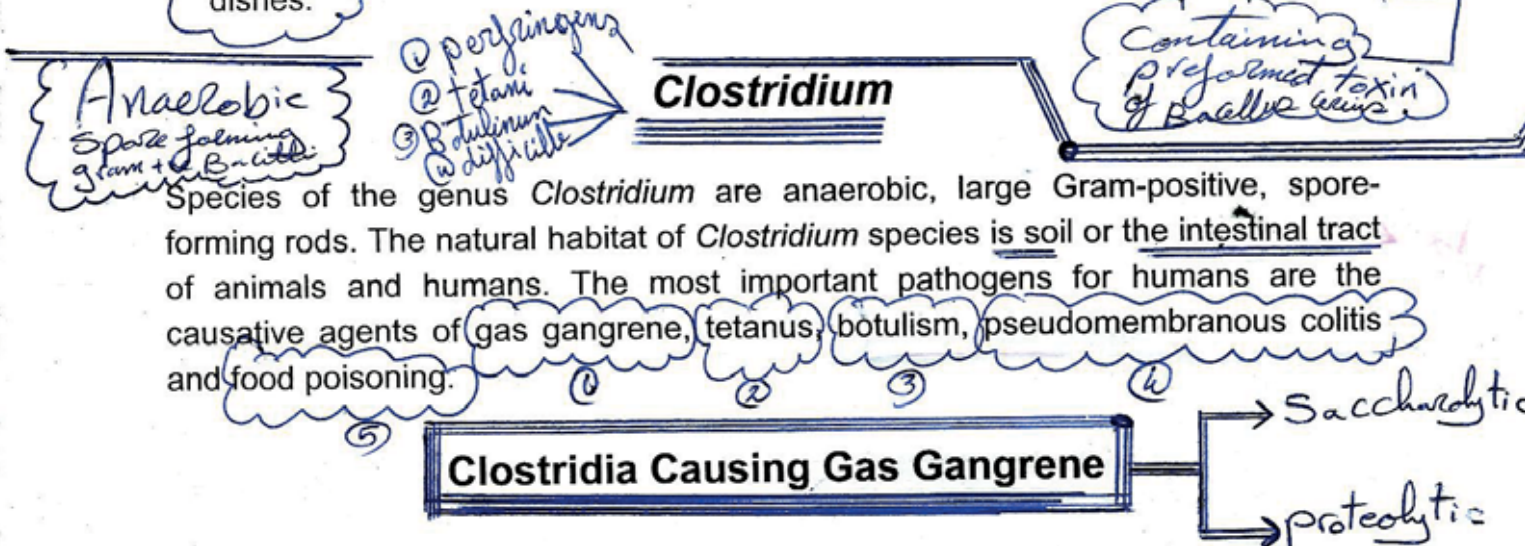
food poisoning by preformed enterotoxin

Compare bet. emetic & diarrheal short long

emetic	diarrheal
short incubat 1-6 h	long incubat 8-16 h

incubation period. This form is caused by a preformed heat-stable enterotoxin (emetic toxin). It is usually associated with improperly refrigerated cooked rice.

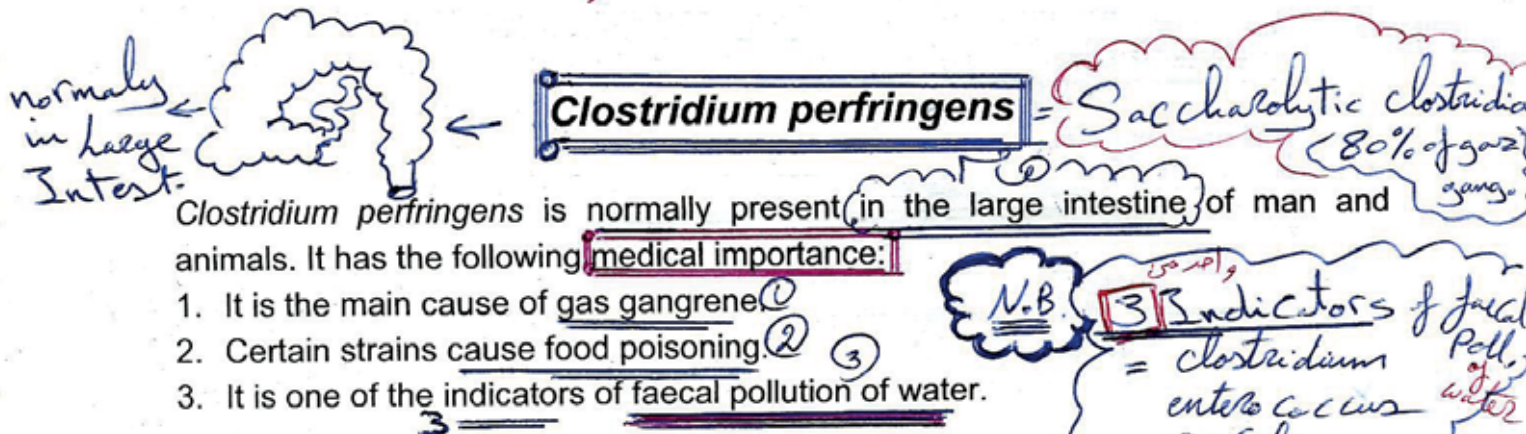
- The "long-incubation" or **diarrhoeal form**: It has an incubation period of 8 to 16 hours and manifests primarily by abdominal cramps and diarrhoea. This type resembles food poisoning caused by *Clostridium perfringens*. This form is mediated by a heat-labile enterotoxin which activates intestinal adenylate cyclase and causes intestinal fluid secretion. It is usually associated with meat dishes.



The primary pathogen can be one or more of various clostridial species:

- Saccharolytic clostridia**
- *C. perfringens* (80%).
 - *C. novyi*.
 - *C. septicum*.
- ferment sugar & produce gas

- Proteolytic clostridia (occasional)**
- *C. bifermentans*.
 - *C. histolyticum*.
 - *C. fallax*.
- proteolytic of protein



Morphology

Gram positive large rectangular bacilli, with oval sub-terminal non bulging spores.

Culture

Anaerobic organism. Colonies on blood agar are surrounded by complete haemolysis.

Biochemical reactions

1. C. perfringens ferments sugars with production of acid and gas.
2. Stormy clot reaction: In litmus milk, the organism ferments lactose with production of acid causing clotting of milk and a large amount of gas which splits this clotted milk into pieces.
3. Nagler's reaction: When cultured on egg yolk agar, C. perfringens colonies are surrounded by opaque zones. This reaction is due to production of lecithinase enzyme (α -toxin). This effect can be inhibited specifically by antibody against α -toxin of C. perfringens.

Virulence factors

1. Alpha-toxin: A lecithinase (phospholipase C) which is lethal and necrotizing. It lyses cell membrane lecithins, disrupting cell membranes and causing cell death.
2. Theta-toxin: oxygen-labile (cytolysin) that also contributes to rapid tissue destruction by several mechanisms. At the site of infection, theta-toxin acts as a cytolysin, causing direct vascular injury and tissue hypoxia. Theta-toxin also mediates the production of shock through induction of inflammatory mediators such as platelet activating factor, tumour necrosis factor, IL-1 and IL-6.
3. Others haemolysins, collagenases, proteases, lipases.....etc.

Gas Gangrene and Related Clostridial Wound Infections

Pathogenesis and clinical findings

All clostridial wound infections occur in an anaerobic tissue environment caused by an impaired blood supply secondary to trauma, surgery, foreign bodies, or malignancy. Clostridial wound infections are usually polymicrobial because the source of wound contamination (faeces, soil) is polymicrobial. Infections are:

A- Gas gangrene or clostridial myonecrosis

Gas gangrene is an acute disease with a poor prognosis and often fatal outcome. Initial trauma to host tissue damages muscle and impairs blood supply. This lack of oxygenation allows the growth of clostridia. Initial symptoms are fever and pain in the infected tissue. As the clostridia multiply, various exotoxins are liberated into the surrounding tissue, causing more local tissue necrosis and systemic toxemia. Infected muscle is discoloured (purple mottling) and oedematous and produces a foul-smelling exudate and gas bubbles which cause crepitations. Clostridial septicaemia, although rare, may occur in the late stages of the disease. Severe shock with massive haemolysis and renal failure is usually the ultimate cause of death. The incubation period varies from 1 to 6 days.

→ infection of muscle → but more gradual onset + no systemic toxemia

B- Anaerobic cellulitis: Like gas gangrene, clostridial cellulitis is an infection of muscle tissue, but it has a more gradual onset than gas gangrene and does not include systemic toxemia. With adequate treatment, it has a good prognosis.

Diagnosis

① → < ② * # start without waiting Laboratory confirmation

Diagnosis is based on clinical findings, Gram staining and culture of clinical specimens. However, treatment must be promptly initiated on a clinical basis without waiting for laboratory confirmation because gas gangrene may spread and cause death within hours.

1. **Specimen** is taken from the depth of the wound and examined rapidly.
2. **Gram stained smear** shows large Gram positive bacilli. There is typically an absence of PMNs due to the clostridial toxins.
3. **Cultivation** on two blood agar plates, one incubated anaerobically and the second incubated aerobically at 37°C for at least 48 hours.
4. **Identification:** Colonies of *C. perfringens* grow only anaerobically and are further identified by morphology, culture characters and biochemical reactions.

Treatment and prevention

1. Correction of the anaerobic conditions combined with antibiotic treatment form the basis for therapy:
 - a. Surgical debridement to expose intact tissues to aerobic conditions.
 - * b. Penicillin is the drug of choice for all clostridial wound infections; chloramphenicol is a second-choice.
- * 2. Hyperbaric oxygen therapy may limit the spread of infection through tissue oxygenation. The patient breaths O₂ at 2-3 atmospheric pressure for 1-2 hours on several successive days.
3. Anti-alpha toxin antiserum: its therapeutic effect is doubtful.
4. Avoiding wound contamination is the single most important factor in prevention of clostridial wound infections. There is no vaccine.

Clostridium perfringens Food Poisoning

C. perfringens is a major cause of food poisoning. The disease results from ingestion of a large number of organisms in contaminated food, usually meat or meat products. *C. perfringens* produces an **enterotoxin**. The incubation period is 8-24 hours after ingestion of contaminated food. Symptoms include diarrhoea, cramps, and abdominal pain and the disease lasts only about 24 hours. No specific treatment is needed. Only fluid therapy is needed to correct the electrolyte imbalance.

CH₁-CH₁₀ ← non motile except 3
 ① tetani
 ② Botulinum
 ③ ~~hesteria~~

Botulinum و Clostridium

Clostridium tetani

Clostridium tetani is the causative agent of tetanus. It is found in soil, especially heavily-manured soils, and in the intestinal tracts and faeces of various animals. The organism is a transient member of the gut flora in 0-25% of humans.

Morphology

C. tetani is a motile Gram-positive rod. It forms a bulging terminal spore that gives the organism a distinctive drumstick appearance.

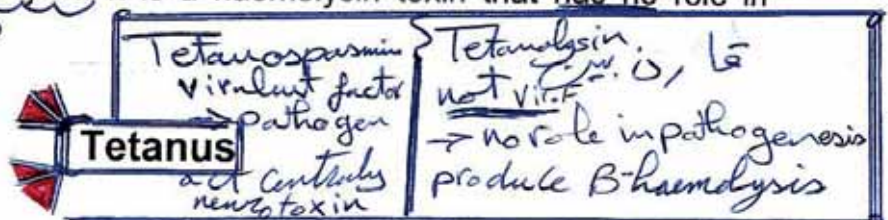
Culture

C. tetani is a strict anaerobe which grows well on simple and cooked meat media. On blood agar, colonies are surrounded by zones of β-haemolysis due to the production of tetanolysin toxin. → not virulent factor because no role in pathogenesis

Virulence factors

C. tetani produces the tetanospasmin toxin, a neurotoxin responsible for the symptoms of tetanus. It is of a single antigenic type although it is produced by different strains of *C. tetani*. It acts centrally at the level of the brain stem and anterior horn cells of the spinal cord.

C. tetani also produces tetanolysin. It is a haemolysin toxin that has no role in pathogenesis.



In spite of the widespread use of the tetanus toxoid for prophylactic immunization, the disease is still a significant problem world-wide. Tetanus is a highly fatal disease; the mortality rates vary from 40% in adults to 90% in the neonates.

Pathogenesis

Mode of transmission = contamination of wound

Most cases of tetanus result from lacerations or small puncture wounds contaminated with *C. tetani* spores. Ingestion of tetanus toxin does not produce the disease.

- The spores germinate in the traumatized tissue where the blood supply is cut off producing an anaerobic environment with low redox potential. A puncture wound can also cause spores to be injected deeply into the tissue, offering anaerobic medium.

The vegetative cells of *C. tetani* grow locally in the necrotic tissue and elaborate tetanospasmin toxin.

* Diphtheria + shigella + cholera + tetanus
 → Tonsils → Colon → Intest. → wound
 So No Blood culture

Tetanus toxin → peripheral nerve end. direct. but must enter CNS via retrograde haematogenous

- Tetanus toxin initially binds to peripheral nerve terminals. It is transported retrograde within the axon until it reaches the central nervous system. The toxin becomes rapidly fixed to gangliosides, at the presynaptic inhibitory motor nerve endings, in brain stem and anterior horn cells of spinal cord. Only a small amount of toxin is needed to produce change.
- Binding of the toxin is an irreversible event, where it is no longer accessible to neutralization by antitoxin.
- The toxin also spreads haematogenously, but it still must enter the CNS via retrograde transport from peripheral neuronal processes.
- **Tetanus toxin blocks the release of the inhibitory neurotransmitters glycine and gamma-aminobutyric acid.** The absence of inhibition permits the simultaneous spasms of both agonist and antagonist muscles, producing **generalized muscular spasms** characteristic of tetanus.
- The incubation period varies from a few days (5-10 days) to several weeks.

Clinical forms

4 forms: Generalized, Neonatal, Localized, Cephalic

Tetanus can be initiated in different ways, resulting in the following forms:

- a- Generalized tetanus (descending tetanus):** Not all the toxin can be absorbed by local nerve endings; therefore, it passes into the blood and lymph with subsequent absorption by motor nerves. The most susceptible centres are the head and neck; the first symptom is usually trismus (lockjaw), with muscle spasms descending from the neck to the trunk and limbs. Spasms often are initiated by environmental stimuli that may be as insignificant as a flash of light or the sound of a footstep. It is seen in unimmunized people.
- b- Neonatal tetanus:** It is seen in newborns when the mother lacks immunity and the umbilical stump becomes contaminated with *C. tetani* spores. It accounts for about half of tetanus deaths in developing countries. It usually results from lack of aseptic technique during birth.
- c- Localized tetanus (ascending tetanus):** Toxin travels along the neural route (peripheral nerves), causing a disease confined to the extremities and seen most often in inadequately immunized people. Localized tetanus may last for months but usually resolves spontaneously.
- d- Cephalic tetanus:** Another unusual form of tetanus which results from head wounds and affects the face. Cephalic tetanus can occur in fully immunized people; the outcome is typically poor.

Host defence and immunity

Innate immunity to tetanus toxin does not exist. In addition, one or more attacks of tetanus do not produce immunity to future attacks. The reason for the lack of immune response may be because: the toxin is potent, and the amount released may be too small to trigger immune mechanisms.

because tetanus toxin is very potent
no innate against tetanus toxin because small amount produce

*

Diagnosis

Why it must be early & fast without waiting Laboratory

Diagnosis is a clinical one, but may need laboratory confirmation. Successful treatment depends on early diagnosis before a lethal amount of toxin becomes fixed to neural tissue. The patient should therefore be treated on a clinical basis without waiting for laboratory data.

to neutralize toxin before becoming fixed to neural tissue which is irreversible. So can't be neutralized

Specimens from the depth of the wound are examined as follows:

1. **Direct Gram-stained smear:** Gram-positive spore-forming bacilli showing drum stick appearance.
2. **Cultivation** on blood agar plate incubated anaerobically at 37°C for 2-3 days.
3. **Identification:** The organism is identified by its morphology and culture characters (see before).

Treatment

Why Human antitoxin? → to avoid hypersensitivity

1. **Human tetanus immunoglobulin (HTIG):** To neutralize the toxin in the blood, tetanus antitoxin is given rapidly. The dose varies from 500 IU in a single intramuscular injection to 3000-6000 IU injected intramuscularly in several sites. Human rather than horse antitoxin is used to avoid hypersensitivity reactions.
2. The organism must be removed by local debridement, after the patient's spasms are controlled by sedation (diazepam).
3. **Metronidazole** is usually administered to kill the bacteria.
4. Supportive measures, such as respiratory assistance and intravenous fluids, are often critical to patient survival.

Prevention and control

active → passive

The elaborated toxin is of a single antigenic type making immunization effective. Prophylactic immunization is the only way to control tetanus because the spores of *C. tetani* are so widely disseminated in nature and cannot, therefore, be avoided. Prophylactic immunization provides the individual with neutralizing antibodies to tetanus toxin in the blood. This could be achieved by:

I. Active immunization:

→ diphtheria P.25 5 1.5

Tetanus toxoid is prepared by treatment of the exotoxin with formaldehyde and addition of aluminum salt. It is given to:

1. **Infants in the first year of life:** The triple vaccine (DPT) is given at the age of 2, 4 and 6 months by intramuscular injection. Booster doses are given at 18 months and upon school entry.
2. **Booster doses are given every 10 years** to maintain immunity. *More frequent boosters are unnecessary and may cause hypersensitivity reactions.*
3. A booster dose is given to wounded individuals with a history of vaccination more than 5 years and less than 10 years ago.
4. People at high risk, e.g. military personnel.
5. **Pregnant females to prevent tetanus neonatorum.** *= neonatal tetanus*

** Why vaccine is only way to prevent tetanus*

Microbiology

II. Passive immunization: HTIG in a dose of 250 IU intramuscularly should be given to wounded individuals:

1. Without a history of active immunization.
2. Without full dose of immunization.
3. With active immunization more than 10 years ago.

ok 1-10 all are motile except 3
 1. Clostridium
 2. Tetanus
 3. Botulinum
 hesterine

Clostridium botulinum

the toxin secreted inactive
 → activated in GIT

ok 1-10 all are motile except 3
 toxin in food poisoning
 not enterotoxin

Clostridium botulinum is widely distributed in soil, sediments of lakes and ponds, and decaying vegetation. Hence, the intestinal tracts of birds, mammals and fish may contain the organism. *C. botulinum* can form part of normal flora of the large intestine of humans. It produces one of the most potent toxins in existence.

Morphology

C. botulinum is a large motile Gram-positive bacillus that forms oval subterminal spores.

اقوى سم في الوجود

Toxin A C. Botulinum

1. most potent poison known ever

Virulence factors

Botulinum toxins: Several toxigenic types of the organism exist, each producing an immunologically distinct form of botulinum toxin. Types A, B and E cause human botulism. Type A toxin is the most potent poison known.

The botulinum toxins are very similar in structure to the tetanus toxin, but differ dramatically in their clinical effects because they target different cells in the nervous system. The botulinum toxin is specific for peripheral nerve endings at the neuromuscular junction where it inhibits the release of acetylcholine.

→ Tetanus
 0-10
 Target

Pathogenesis of botulism

a- Food-borne botulism:

- Spores, widespread in soil, contaminate vegetables and meat. When these foods are canned without adequate sterilization, spores survive and germinate in the anaerobic environment.

ingestion of preformed food toxin in unsterilized canned food so spore survive

- Toxin is produced within the canned food and ingested **preformed**. The highest-risk foods are vegetables such as green beans and mushrooms, smoked and salted fish, and commercially canned salmon.

- The toxin is activated by proteases in the gastric fluid and by gastric acidity. The toxin is absorbed in the intestine and is transported systemically via the bloodstream to reach the peripheral neuromuscular synapses.

The toxin binds to the neurone and prevents the release of acetylcholine across the synaptic cleft. Thus, it produces paralysis of the motor system. Botulinum toxin is quick acting so the incubation period may be as short as 12 hours.

Wabli*
 Preformed toxin inactive
 ↓
 activated in GIT
 ↓
 Neurotoxin



- Once the toxin is fixed to the tissue, its actions are very difficult to reverse with antitoxin treatment. Antitoxin is only effective if it binds to the toxin before the toxin binds the neuromuscular junction (within 12 hours after ingestion).

The primary symptom is weakness or **flaccid paralysis**. The cranial nerves are affected first (blurred vision, inability to swallow, difficulty in speech), followed by a descending, symmetric paralysis of motor nerves. Nausea and vomiting are not usually prominent. No fever is apparent. Death is usually caused by respiratory failure.

b- Infant Botulism: In contrast to food poisoning caused by ingestion of preformed toxin, infant botulism results from germination of spores in the gastrointestinal tract where vegetative cells replicate and release the botulinum toxin. The disease occurs in infants between 2 weeks and 6 months of age. The bacterium can establish itself in the bowel of infants before the establishment of competing intestinal flora. The disease is characterized by constipation and weak sucking ability and generalized weakness. Almost all cases of the disease recover.

Diagnosis

Diagnosis is a clinical one but may need laboratory confirmation. The most direct and effective way to confirm the clinical diagnosis of botulism in the laboratory is to demonstrate the presence of toxin in the serum or faeces of the patient or in the food which the patient consumed.

Culturing of specimens takes 5-7 days. The organisms can be easily recovered using cooked meat medium. Organism in culture is identified by its morphology and biochemical characters.

Treatment

A potent trivalent (A, B, E) antitoxin is available; it must be promptly administered IV to individuals known to have ingested food with botulinum toxin. Normally there are no organisms, therefore, there is no reason to give antibiotics except in infant botulism.

Prevention

- The most important aspect of botulism prevention is proper food handling and preparation.
- Because the toxin is heat-labile, boiling for 10 minutes or intense heating (cooking) of contaminated food will inactivate the toxin.
- Swollen cans must be discarded.

Why no need to Antibiotic in adult in contrast to infants?
→ Because there is no organism of infants? But toxins only

Clostridium difficile

Part of normal flora

12-25% Antibiotic associated Colitis
diarrhoea

C. difficile is an anaerobic spore-forming, Gram-positive bacillus, which can be part of the normal intestinal flora. *C. difficile* is the most common cause (15-25%) of antibiotic-associated colitis, namely **pseudomembranous colitis** and antibiotic-associated diarrhoea (**superinfection**).

Virulence factors and pathogenesis

Toxin A, B
A, B

- Cause**
- When the normal flora is suppressed, due to overuse of antibiotics *C. difficile* grows and produces two toxins. Toxin A is an enterotoxin that causes fluid accumulation in the bowel; toxin B is a potent cytotoxin. Both toxins result in alterations in the actin microfilaments responsible for cytoskeleton of the cells. This can cause loosening of the tight junctions between the colonic epithelial cells and, hence, diarrhoea. Both toxins have effects on leukocytes that include induction of tumour necrosis factor, IL-1 and IL-6 that contribute to the inflammatory response, i.e. diarrhoea that may progress to pseudomembranous colitis.
- Incubation period: Diarrhoea may occur 4-10 days after start of antibiotics.

Diagnosis

How to differentiate *C. difficile* from transient diarrhoea

Pseudomembranous colitis can be differentiated from the transient diarrhoea that occurs as a side effect of many oral antibiotics by detection of *C. difficile* toxins in stools either by a cell cytotoxicity assay or by ELISA.

Treatment

1. Withdrawal of the causative antibiotic.
2. Treatment with anti-*C. difficile* drugs: Oral vancomycin or metronidazole may be required in an extended course to prevent recurrence (occurs in 15 to 20% of patients).
3. Correction of dehydration and electrolyte imbalance.
4. Antidiarrhoeal agents should NOT be taken.

* Mode of transmission of *Clostridium difficile*
= Endogenous because it's normally part of flora but pathogenic if over dose Antibiotics

verruent. Angina @
diphtheria @ }
C. difficile @ }
Shigella dysenteriae @ } Intest. Colon

Pseudomembranous

Chapter 8

NON SPORE-FORMING ANAEROBES

These obligate anaerobes form the major part of normal microbial flora in the mouth, GIT and female genital tract. They are also incriminated in a wide variety of infections. Most infections with these organisms are of endogenous origin.

G -ve BACTEROIDES: These are Gram-negative, capsulated, highly pleomorphic bacilli. The capsule is the virulence factor. They constitute more than 90% of the normal flora of the stools. B. fragilis is the commonest pathogen. It is associated with intra-abdominal and soft tissue infections below the diaphragm. It is the most common anaerobe found in bacteraemia and, occasionally, in infections of the head and neck. Most strains are resistant to penicillin but sensitive to metronidazole, clindamycin and chloramphenicol.

G -ve FUSOBACTERIUM: They are large Gram-negative fusiform or cigar-shaped bacilli. They are frequently isolated from dental or brain abscesses. In association with spirochaetes, they produce fusospirochaetal disease in the oral cavity.

G +ve PROPIONIBACTERIUM: They are Gram-positive bacilli, having the typical morphology of diphtheroides. Their metabolic products include propionic acid from which the genus name is derived. P. acne is normally present in human skin, hair, oropharynx and GIT. It is the major contributor to the complex pathogenesis of acne vulgaris.

G +ve LACTOBACILLUS: The genus includes Gram positive, nonspore-forming, non motile bacilli. They are strongly acid producing (acidogenic) by fermentation of carbohydrates. They can survive and grow in acidic media (aciduric) with pH 4-5. Lactobacilli are normal commensals of the oral cavity, intestine and vagina. In the last two sites, they have a beneficial protective (probiotic)* effect due to acid production which inhibits colonization with pathogenic organisms. The commonest species of medical importance include: (1) L. odontolyticus present in the mouth and is involved in dental caries, (2) L. acidophilus present normally in milk, saliva and intestine. Living cultures are used in treatment of intestinal disturbances, and (3) L. doderleins present normally in the vagina and is responsible for its acidic pH.

* The **Probiotics** are live, nonpathogenic bacteria that may be effective in the treatment or prevention of certain diseases. They either exclude the pathogen from binding sites on the mucosa or enhance the immune response against the pathogen, e.g. L. rhamnosus is used to reduce cases of nosocomial diarrhea.

Chapter 9

MYCOBACTERIUM

The genus *Mycobacterium* comprises **acid-fast bacilli (AFB)** that have caused disease in humans since the recorded history (lesions in Egyptian mummies). *Mycobacterium tuberculosis* is the principal causative agent of tuberculosis.

Characteristic features of the genus

- 1. Acid-fastness:** Acid-fastness means that once organisms are stained with certain dyes such as carbol-fuchsin (in Ziehl-Neelsen method) or auramine-rhodamine (in fluorescent acid-fast stain), they retain the dye firmly and resist decolorization even by acid-alcohol or strong mineral acids. Acid-fastness is due to the high lipid (mycolic acid) content of the cell wall.
- 2. Slow rate of growth:** *Mycobacteria* have a longer generation time and, consequently, a slower rate of growth than ordinary bacteria. This growth rate varies among different species so that it permits their grouping into slow and rapid growers. Slow growers require more than 7 days to produce visible colonies on solid media while rapid growers require less than 7 days.
- 3. Pigment production** in light and dark

* G.R. Mycobact. is acid fast Bacilli

decolorization with high lipid content of cell wall

slower 7 days relative rapid growers < 7 days

produce pigment in light and dark
not produce pigment in light and dark

Members of the genus *Mycobacterium*

- 1. *Mycobacterium tuberculosis* complex:** *M. tuberculosis* and three very closely related species (*M. bovis*, *M. africanum* and *M. microti*) compose what is known as the *M. tuberculosis* complex. They are also called **tubercle bacilli**. They can cause tuberculosis in man and animals. Humans are the only reservoir for *M. tuberculosis* whereas both cows and humans are reservoirs for *M. bovis*.
- 2. Nontuberculous mycobacteria (NTM),** e.g. *M. avium* and *M. fortuitum*.
- 3. *Mycobacterium leprae*:** the causative organism of leprosy.
- 4. Saprophytic mycobacteria species:** rarely incriminated in human diseases.

P.47

Mycobacterium tuberculosis

Morphology

M. tuberculosis (Mtb) are slender acid-alcohol-fast bacilli that may appear curved. They appear pink in a blue background when stained with the Ziehl-Neelsen or Kinyoun method. When stained with the auramine-rhodamine, AFB fluoresce orange yellow in a black background. In patients under treatment, it may show irregular staining or beaded appearance. Chains of cells in smears prepared from

Stains

Ziehl-Neelsen or Kinyoun



cultures may form distinctive serpentine cords. It is believed that this appearance is due to cord factor associated with virulent strains.

Cultural characters

M. tuberculosis requires special media for growth:

- ① Lowenstein-Jensen (L-J) medium which is a selective egg-based medium. not agar based med.
- ② Middlebrook's medium which is a synthetic enriched medium. It can be rendered selective by addition of antibiotics. It may be fluid (broth) or solid (agar-based).

M. tuberculosis is a strict aerobe. Its growth is enhanced by addition of 5 to 10% CO₂.

Colonies appear after about 2-4 weeks of incubation at 37°C and may be delayed to 8 weeks during primary isolation from clinical specimens. Serpentine cords can best be detected in broth medium.

Biochemical reactions

Biochemical tests (e.g. niacin production and nitrate reduction) are used for the definitive identification of mycobacteria along with culture characters (growth rate and pigment production). Human T.B. Cause

Cell wall structure

The cell wall of the tubercle bacilli is unique to the *Mycobacterium* species. A layer of mycolic acids and glycolipids lies external to the peptidoglycan layer. The peptidoglycan layer determines the shape of the cell and is similar to the peptidoglycan of Gram-positive bacteria. Tubercle bacilli comprise several substances that are thought to contribute to disease production:

1. Mycolic acids: are long chain fatty acids containing 60 to 90 carbons.
2. Cord factor: is a cytotoxin.
3. Mycobacterial sulfolipids: inhibit phagolysosome formation.

* GR.T.B. resist intracellular killing? bec sulfolipid inhibits phagolysosome formation

The high concentration of lipids in the cell wall of Mtb has been associated with:

- a- Impermeability to stains and dyes.
- b- Resistance to many antibiotics.
- c- Resistance to killing by acidic and alkaline compounds.
- d- Resistance to osmotic lysis via complement deposition.
- e- Ability to survive inside macrophages and induce CMI response.

Susceptibility to physical and chemical agents

1. Tubercle bacilli are killed by:

- Heating at 55°C for 1 h, autoclaving, pasteurization and sunlight.
- A number of chemical disinfectants (intermediate level disinfection) such as ethyl and isopropyl alcohols and chlorine.



2. They survive for many weeks in moist conditions in the dark and many days when dried in sputum smeared on clothing and in dust.
3. They are resistant to acids and alkalis, an important feature, which is used in cultivation procedures from contaminated specimens.

Human Tuberculosis

G.R. Tuberculosis (TB) is a major cause of death in the world. The number of cases with TB is increasing after it was decreasing before AIDS pandemic, a condition called resurgence of TB. This situation is very serious not only because of the rising number of cases but also because many of such bacteria that cause the new outbreaks are usually multi-resistant to the anti-tuberculosis drugs.

Pathogenesis and clinical manifestations

A. Mode of infection: The commonest mode of infection is inhalation of droplet nuclei carried by air (aerosol infection) from a patient with open pulmonary tuberculosis. These droplet nuclei reach the terminal alveoli where they are engulfed by alveolar macrophages. The majority of these bacilli are destroyed or inhibited. Ingestion of milk contaminated with *M. bovis* may cause intestinal infection.

B. Local lesion: The remaining bacilli are able to **survive and multiply** intracellularly by inhibiting fusion of phagosomes and lysosomes. The accumulating bacilli kill the initial alveolar macrophages. Two weeks later, the bacilli are transported via lymphatics to the regional lymph glands.

C. Transient bacteraemic (bacillaemic) phase: A few days later, organisms leave the lymph glands to the blood stream. This bacillaemic phase of the infection leads to dissemination of organisms throughout the body to more distant tissues e.g. the apices of the lungs, the kidneys, the brain, and bone.

D. CMI response, activation of macrophages and granuloma formation:

- CMI response, which is the main immune mechanism against mycobacteria, is initiated about 2-10 weeks after infection.
- Specific Th1 cells recognize infected macrophages and release cytokines (particularly IFN- γ) that activate macrophages and attract more macrophages to the site of infection.
- In addition, IL-12 (produced by activated infected macrophages) promotes the differentiation of Th cells into Th1 cells augmenting the production of IFN- γ .

- When mycobacteria resist the microbicidal effect of the activated macrophages, a characteristic localized inflammatory response called a **granuloma (tubercle)** develops. This serves to isolate pathogens that resist intracellular destruction.



The inability of *M. tuberculosis* to multiply within these tubercles is also due to the low pH and anoxic environment (Mtb is a strict aerobe), however, it can survive in small numbers in a relatively **dormant state** (latent tuberculosis infection). This situation is due to a balanced state of host-parasite relationship.

* G.R. of dormant state as 3
 ① Immune system
 ② ↓ O₂
 ③ ↓ pH

E. The host-parasite interactions:

- Latent tuberculosis infection:** The above mentioned events can all occur without development of symptoms. This occurs in about 90% of the infected people. Individuals with this initial tuberculosis infection are tuberculin positive.
- Tuberculosis disease:** In the remaining 10% of people, TB bacilli overcome the immune system and begin to multiply, resulting in the progression from TB infection to TB disease (Table 3). Symptoms of disease include general malaise, fatigue, night sweat and fever, along with persistent cough and bloody sputum.

N.B.:

- Under certain conditions, e.g. immunosuppression, disturbance of the host-parasite balance may lead to reactivation of latent tuberculosis infection and development of tuberculosis disease.
- Tuberculosis may affect other systems, e.g. tuberculous meningitis, lymphadenitis, renal and intestinal tuberculosis.

Table (3): Latent tuberculosis infection versus disease.

Characters	TB Infection	Pulmonary TB disease
Mtb present	Yes	Yes
Tuberculin test	Positive	Positive
Sputum smears and cultures *	Negative -ve *	Positive *
Symptoms *	No symptoms *	Cough, fever, weight loss *
Communicability *	Not infectious *	Often infectious before treatment *
Case definition *	Not a case of TB *	A case of TB *

Laboratory diagnosis of open pulmonary tuberculosis

The laboratory diagnosis of tuberculosis disease depends on the detection and isolation of *M. tuberculosis* complex or one of its members from clinical specimens.

(A) Specimens include sputum, bronchoalveolar lavage, etc. Three early morning sputum specimens collected on consecutive days, from a deep productive cough, give the best results.

(B) Processing of sputum by liquefaction, decontamination and concentration: This procedure is applied to sputum as well as other specimens that contain organisms other than mycobacteria. It is done to prepare such specimens for culture and, sometimes, for smear examination. It enhances detection of the AFB by culture and stained smears because:

Important Liquefaction of sputum releases trapped mycobacteria from tenacious specimens.

- The mycobacteria in the liquefied specimen can then be concentrated in small sediment by centrifugation.
- Decontamination kills normal flora allowing obvious growth of the slowly growing mycobacteria.

Mechanism The most commonly used method is the **N-acetyl-L-cysteine - sodium hydroxide method**. It is based on the ability of NaOH, at a certain concentration, to inhibit organisms other than mycobacteria. The mucolytic agent N-acetyl-L-cysteine (NALC) is combined with NaOH to help liquefaction of sputum.

GR. using N-acetyl cysteine
Liquefaction
Inhibit other
↑ Conc. in centrif.

C. Direct detection

1- **Smears** prepared directly from sputum or sediment after concentration are subjected to one of the following **acid-fast staining methods**:

- Ziehl-Neelsen (Z-N) or Kinyoun method**, using the carbol-fuchsin dye. In Z-N method, heat is used to help penetration of carbol-fuchsin. In Kinyoun method, heat is not used but phenol is added to the carbol-fuchsin. Under the ordinary light microscope, AFB appear pink in a blue background.
- Fluorochrome staining with the auramine-rhodamine stain**. Under the UV microscope, AFB fluoresce orange yellow in a black background.

افضل دليل Detection of AFB in stained smears may provide the earliest clue of pulmonary TB. Smear examination is an easy and quick procedure, compared to culture results which are available only after several days or weeks. Fluorochrome staining, though more expensive, is faster and more sensitive than the traditional Z-N method.

* AFB = Acid fast Bacilli

* what is advantage of $\downarrow$ +ve AFB?

A positive AFB sputum smear has the following advantages:

- Early diagnosis which allows rapid patient care. The result should be available to the clinician within 24 hours.
- Monitoring the infectiousness of the patient and effectiveness of anti-tuberculosis therapy.

2- **Nucleic acid amplification tests:** PCR can be used for rapid (same day) detection of *M. tuberculosis* in sputum samples.

D. Cultivation: Definitive diagnosis of TB disease is achieved only by a positive culture. One of the following culture media can be selected; inoculated by sediment of processed sputum and incubated in 5 to 10% CO₂ at 37°C:

1- **Conventional culture media:** Culture on L.J. medium allows study of colony morphology, pigment production and growth rate.

2- **Fluid medium systems:** Middlebrook broth is used as the base for the following systems:

a. **Bactec AFB system:** Bactec medium contains radio-labelled palmitate as the sole carbon source. As *Mtb* multiplies, it breaks down the palmitate and liberates ¹⁴C-labelled CO₂ that can be detected.

b. **Mycobacteria Growth Indicator Tube (MGIT):** It contains a fluorescence sensor that fluoresces (upon exposure to UV light) when oxygen is depleted from the medium due to bacterial growth.

The major advantage of culture on these fluid systems is that it allows detection of growth in 4 to 14 days.

E. Identification:

- 1- Colonies appear after about 2-4 weeks of incubation or may be delayed to 8 weeks.
- 2- Colonies of *M. tuberculosis* are nonpigmented, rough, slowly growing and show AFB. *M. tuberculosis* is positive for nitrate reduction and niacin production.

F. Tuberculin test (see below). *detects delayed hypersensitivity response to previous exposure*

Laboratory diagnosis of extrapulmonary tuberculosis

Diagnosis of extrapulmonary TB such as tuberculous meningitis, lymphadenitis, renal and intestinal tuberculosis is performed as mentioned for pulmonary TB but by collecting the appropriate specimens. However, specimens collected from normally sterile sites, e.g. CSF, do not require decontamination.

So meningitis not diagnosed by CSF decontamination because it's sterile

Tuberculin Skin Test (TST)

main test used to diagnose Latent tuberculosis infection

Principle: The tuberculin test is a skin test that detects delayed hypersensitivity response to previous exposure of the host to tubercle bacilli. It is one of the main tests used to diagnose latent tuberculosis infection. Purified protein derivative (PPD) is the antigen used in the tuberculin test.

(PPD) = antigen in tuberculin test

Underlying mechanism: As a result of previous exposure of the host to tubercle bacilli, Th1 cells are sensitized, activated and clonally expanded. In positive reactors, the injected tuberculin substance stimulates the presensitized Th1 cells to secrete cytokines which recruit inflammatory cells particularly macrophages. The result is a **raised, indurated** area around the site of injection. No reaction is seen in people who have not been sensitized to TB.

Technique:

- 0.1 ml of PPD containing 5 tuberculin units (TU) is injected intradermally in the skin of the anterior aspect of the forearm (Mantoux method).
- The result is read after 48-72 h by palpating for the presence of **induration**. The diameter of the induration is measured in millimeters. The induration is what is measured, NOT the erythema (red area).

Interpretation of the tuberculin test

→ categorized by risk factors
5-10-15 mm system

The reaction was originally regarded as either "positive" or "negative". However, recently, reactions have been categorized by different criteria (risk factors) depending on the circumstances of the patient. This is the so-called "5-10-15 millimeter system" (Table 4).

Table (4): Interpretation of the tuberculin skin test

 An induration of 5 or more millimeters	 An induration of 10 or more millimeters	 An induration of 15 or more millimeters
considered positive for: 1. People who have had <u>TB disease before</u> 2. <u>Close contacts of people with infectious TB</u> 3. <u>People with HIV infection</u>	considered positive for: 1. People in <u>endemic areas</u> where TB is common 2. People with certain medical conditions such as <u>diabetes</u> 3. <u>Unvaccinated children younger than 4 years old</u>	considered positive <u>even in absence of any risk factor for TB</u> MCQ 120

↑ Risk factors 45

* why Tuberculin test not diagnostic?
bec. $\begin{cases} 2 \text{ false -ve} \\ 2 \text{ false +ve} \end{cases}$

False negative reactions

1. **Anergy**: Anergy is the inability to react to TST because of a weakened immune system, e.g. severe TB disease, HIV infection, or cancer.
2. **Recent TB infection**: After exposure, it takes 2 to 10 weeks for tuberculin test to become positive.

False positive reactions

1. Infection with nontuberculous mycobacteria (NTM) due to cross-reaction with *M. tuberculosis* antigens.
2. **Vaccination with bacille Calmette-Guérin (BCG)**: After BCG vaccination, tuberculin skin test remains positive for up to 5 years.

Treatment regimens of tuberculosis

The first-line drugs in the treatment of tuberculosis are isoniazid (INH), rifampin, pyrazinamide, ethambutol and streptomycin. Second-line drugs are ciprofloxacin, para-aminosalicylic acid, ethionamide, cycloserine, capreomycin, kanamycin, amikacin and rifabutin; these drugs are only used if resistance occurs during administration of first-line drugs.

1st Line $\begin{cases} \text{isoniazide} \\ \text{rifampin} \\ \text{pyrazinamide} \\ \text{ethambutol} \\ \text{streptomycin} \end{cases}$
2nd Line $\begin{cases} \text{ciprofloxacin} \\ \text{para-aminosalicylic acid} \\ \text{ethionamide} \\ \text{cycloserine} \\ \text{capreomycin} \\ \text{kanamycin} \\ \text{amikacin} \\ \text{rifabutin} \end{cases}$

resistance to 1st Line $\rightarrow$ 2nd Line

To be successful, the treatment should fulfill the following requirements:

1. **Combination therapy**: Combination of 4 drugs or more is essential to reduce the drug toxicity and to prevent the emergence of drug resistant mutants.
2. **Duration of treatment**: The treatment should be continued for at least 6 months. because it's bacteria is dormant

* Because of patients non-compliance, a regimen recommended by WHO known as directly observed therapy short course (DOTS), is thought to be effective for such patients.

M. tuberculosis isolates should be tested for drug resistance, as early as possible in order to ensure appropriate treatment. Multidrug-resistance means MTB resistant to both isoniazid and rifampin. It is a serious situation that presents difficult treatment problems.

Prevention and Control

A. General measures

- Early case finding and effective treatment.
- Applying proper infection control measures in hospitals.
- Avoid overcrowding.
- Pasteurization of milk.

B. Treatment of latent tuberculosis: Isoniazid (INH) is taken for 6-9 months in:

- Recent converters: asymptomatic patients whose tuberculin skin test recently converted to positive.
- Children or health care workers exposed to open case of pulmonary tuberculosis.
- Tuberculin-positive individuals who undergo immunosuppression.

C. BCG vaccine: a live attenuated vaccine prepared from *M. bovis*, is given to newborns **intradermally** in the skin over the left deltoid region. The protective efficacy of BCG vaccine is a subject of controversy and many attempts have been made to develop a better vaccine.

Non-Tuberculous Mycobacteria (NTM)

no person to person
only environmental transmit

Nontuberculous mycobacteria (NTM) include those *Mycobacterium* species that are not members of the *Mycobacterium tuberculosis* complex; hence the use of the terms "nontuberculous mycobacteria" or "mycobacteria other than tuberculosis" (MOTT). Most NTM have been recovered from tap water, water of haemodialysis units and drinking-water distribution systems.

General features of NTM

مميز قوش عن بكتريا الشكل

1. Most NTM are morphologically indistinguishable from *M. tuberculosis*.
2. Most NTM grow well on media used to grow *M. tuberculosis*.
3. Growth characteristics (rate of growth and pigment production) are used for preliminary grouping.
4. NTM generally cause opportunistic infections. → on low immune individuals
5. Person to person transfer does not take place. Transmission is from environmental sources.
6. NTM are generally more resistant to drugs used for treatment of TB. Combinations may have to be used (Rifabutin, clarithromycin and azithromycin).
7. NTM show high resistance to wide range of disinfectants.

Table (5): Examples of important NTM and the diseases they produce

Microorganism	Disease produced
Slow growers <i>M. avium</i> Complex (MAC): <i>M. avium</i> , <i>M. intracellulare</i>	Pulmonary disease, lymphadenitis in children, and disseminated infections in AIDS patients.
<i>M. kansasii</i> <i>M. ulcerans</i>	Chronic lung disease resembling classical TB. Ulcerating infections of the skin (Buruli ulcer).
Rapid growers <i>M. fortuitum</i> Complex: <i>M. abscessus</i>	Chronic lung disease, post-traumatic wound infections, abscesses, disseminated cutaneous disease and nosocomial bacteraemia associated with contaminated haemodialysis equipment.
<i>M. chelonae</i>	Disseminated nodular skin disease.

* ابطأ
معد
19
slow
19
Rapid
فقط
والبكتريا
البيوت
داوس

M. chelonae

Mycobacterium leprae

افلام الخلفى الزرقاء

Mycobacterium leprae (Hansen's bacillus), the causative agent of leprosy, was discovered by Hansen in 1873.

Morphology

G.R. Why we use Modified Ziehl-Neelsen not normal in *M. leprae*?

Mycobacterium leprae vary in the degree of acid- and alcohol-fastness in comparison with *M. tuberculosis*. Therefore, the use of the **modified Ziehl-Neelsen** method, in which the decolourizing agent is modified, is necessary to avoid overdecolourizing the AFB. A modified Z-N stain utilizes 1% acid alcohol as a decolourizer. *M. leprae* closely resembles *M. tuberculosis* in size and shape. It occurs chiefly in bundles (globi) within the lepra cells.

Culture in vitro

M. leprae has not yet been successfully cultured in vitro.

Culture in vivo

1. **The nine-banded armadillo**: The nine-banded armadillo can be infected with *M. leprae* and this animal has become the main source of *M. leprae* for biochemical and immunological research including development of a vaccine.
2. **Normal mice**: Inoculation of the specimen into the hind foot-pads of mouse is used to test the sensitivity of the bacilli to new drugs.

Leprosy

Causative organism: *M. leprae*
Mode of Transm.: unknown
But require Prolonged Contact

Leprosy (Hansen's disease) has struck fear into human beings for hundreds of years. Although *M. leprae* was discovered by Hansen in 1873, treatment for leprosy only appeared in the late 1940s with the introduction of dapsone. By the early 1980s, Leprosy bacilli resistant to dapsone appeared.

Pathogenesis and clinical manifestations

The exact mode of transmission is not known. However, infection requires prolonged and close contact with patients. Leprosy is a chronic infectious disease that primarily affects cooler surface areas of the body, such as the skin, ear lobes, mucosa of the nose, mouth and upper respiratory tract and also the eyes. There is usually some degree of irreversible nerve damage. The pathogenesis of leprosy appears to derive from: 1) the ability of *M. leprae* to survive and replicate within the phagosomes of macrophages, in nerve cells and other host cells, and 2) the consequent immune response to the organism. The disease is not a single clinical entity but presents in two basic forms: **tuberculoid leprosy (TL)** and **lepromatous leprosy (LL)**, with many intermediate forms inbetween.

A Tuberculoid leprosy

- Cell-mediated immune response predominates and forms granulomas resulting in the destruction of most of the mycobacteria. So only few AFB remain in the tissues (paucibacillary or PB leprosy).
- Lesions are few and mainly in the form of hypopigmented maculo-anaesthetic skin lesions.
- Although skin and peripheral nerves are damaged, TL progresses slowly, sensory loss is mild and patients usually survive. Spontaneous regression of tuberculoid leprosy occurs in over 90% of cases.

B Lepromatous leprosy (LL)

- Cell-mediated immune response is depressed. Although humoral response is predominating, it is not protective as the organism is intracellular. The AFB are widely disseminated in macrophages and lesions usually contain large numbers of AFB (multibacillary or MB leprosy).
- The AFB form clumps and occur as intra- and extracellular masses known as globi.
- Lesions are mainly nodular and may form on the face. As the disease progresses, the nose may collapse giving the characteristic lionine facies.
- LL is the more severe form and progresses rapidly. There is a marked sensory loss due to extensive nerve damage.

The development of TL or LL depends on the balance of Th1 and Th2 cells. In TL, the immune response is characterized by a Th1-type response with delayed-type hypersensitivity and a cytokine profile consisting of high levels of IL-2, IFN- γ and TNF- β . In LL, there is a Th2-type response with high levels of IL-4, IL-5 and IL-10. This cytokine profile explains the diminished cell-mediated immunity and increased production of serum antibody in LL (Table 6).

Table (6): Comparison between tuberculoid leprosy and lepromatous leprosy

	Tuberculoid leprosy	Lepromatous leprosy
Immune response <i>Handwritten: CMI = Cell mediated Imm. AMI = Antibody med. Imm.</i>	- Predominant CMI (skin infiltrated with Th1 cells)	- Diminished CMI - Predominant AMI (high antibody levels) <i>Handwritten: bec. intracellular</i>
T helper-type response	Th1	Th2
Lepromin test	Positive <i>Handwritten: bec. CMI dominant</i>	Negative <i>Handwritten: GR: no CMI</i>
Cytokine profile	IL-2, IFN- γ and TNF- β	IL-4, IL-5 and IL-10
Number of AFB in tissues	Few (<i>Handwritten: Acid Fast Bacilli</i>)	Abundant (<i>Handwritten: Acid Fast Bacilli</i>)
Lesions	Macular skin lesions	Nodular skin lesions
Disease progress	Slow	Aggressive
Prognosis	Good (<i>Handwritten: Better</i>)	Bad
	<i>Handwritten: Negation 90% of 49 cases</i>	<i>Handwritten: nerve damage</i>
	<i>Handwritten: form granuloma</i>	<i>Handwritten: Though humoral AMI</i>



Laboratory diagnosis

1. The diagnosis of leprosy is essentially a clinical one, supported by finding AFB on slit skin smears or in skin biopsy specimens, or scrapings from the nasal mucosa. Smears can be stained with modified Z-N method.
2. PCR assays. *→ not diagnostic but →*
3. **The Lepromin skin test:** The test is an indicator of the ability of the host to raise a cell-mediated immune response to *M leprae*. Lepromin is a heat-killed suspension of *M. leprae* prepared from armadillo tissue. *Medicine* The test itself is of no diagnostic, but of prognostic value. It is positive in tuberculoid leprosy and negative in lepromatous leprosy.

Treatment

= Multidrug = rifampicin + clofazimine + dapsone
to prevent resistance of using single drug

Multidrug therapy is the strategy designed by the WHO to eliminate leprosy. The drugs used are rifampicin, clofazimine and dapsone. Among these, rifampicin is the most important antileprosy drug and, is therefore, included in the treatment of both forms of leprosy. Use of only one drug will always result in development of resistance. Nerve damage and deformities are irreversible.

Chloroquine *Acute*
 @ 10 Gonorrhea
 @ 2 Leprosy
 @ 3 Vaginitis } Direct detection is enough *smear*

@ widal
 @ lepromin test is not diagnostic test bec.
 can be false +ve or -ve

Lact. form. → E. coli
→ Klebsiella

Lact non form. → E. coli
→ Salmonella

Chapter 10

Ch 10 - ch 18
G-ve Bacilli
non spore forming

ENTEROBACTERIACEAE

Gram -ve bacilli
Facultative anaerobe
oxid. -ve
ferment D Glucose
red. nitrate → nitrite

The Enterobacteriaceae family is a large group of facultative anaerobic, nonspore-forming, Gram-negative bacilli. The natural habitat is the intestinal tract of humans and animals. Some members are widely distributed in the environment as well in water and soil, and on plants.

It is classified into many genera and species on the basis of biochemical reactions, especially fermentation of carbohydrates, as well as DNA-DNA hybridization.

General features: Members of Enterobacteriaceae share features that help differentiating them from other Gram-negative bacilli.

1. **Facultative anaerobes:** They grow both aerobically and anaerobically, and grow well on MacConkey agar.
2. **Oxidase negative.**
3. **Ferment D-glucose.** Fermentation of other sugars is variable; lactose fermentation is an important differential character.
4. **Reduce nitrate to nitrite.**

N.B. *Pseudomonas aeruginosa* and *Bacteroides* species which are Gram-negative bacilli normally found in human intestines are considered non-Enterobacteriaceae. This is because *P. aeruginosa* is an obligate aerobe and usually oxidase positive, whereas *Bacteroides* is a strict anaerobe.

Medical importance

A. Opportunistic pathogens: Most Enterobacteriaceae, such as *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia* and *Proteus* genera, are commensals and may cause opportunistic infections.

B. Enteric pathogens: *Salmonella*, *Shigella*, and *Yersinia* genera, and certain strains of *Escherichia coli* primarily cause intestinal infections and systemic diseases (e.g. typhoid fever caused by *Salmonella Typhi*).

Escherichia coli

Importance of E. coli
most predominant in large Intest
anaerobe

Imp of Escherichia coli is the most predominant facultative anaerobe in the large intestine of man and is part of the normal bowel flora. Although there are certain pathogenic strains which, under special circumstances, are responsible for serious intestinal and extraintestinal diseases, *E. coli* as a normal flora provides protection against colonization by harmful microorganisms. Also, *E. coli* is used as one of the important indicators of faecal pollution of water since it is constantly found in human and animal faeces.

G.R. E. coli used as indicator? → bec.
51 stool Intestine → 99% die

Wikipedia

It is more blessed to give than to receive
 حرف الكافى عا وك
 حرف الكافى عا وك

قادر
 حرف الكافى عا وك

ch10 - ch18
 Gr - ve bacilli
 Can't differentiated by Morphology

Morphology ①
 Gram-negative bacilli, usually motile and some are capsulated.

Cultural characters
 E. coli grows on MacConkey agar producing rose pink colony due to **lactose fermentation**.

pepton + bile salt + agar after heat + Lactose + pH indicator
 nutrient to selective to solidify to defenital red neutral

* Gr. E coli on Mac. produce rose pink bec. Lactose fermenter → gas + acid

* 5s sugar fermenter

Biochemical reactions

Most E. coli strains are:

1. Fermenters to glucose, lactose, maltose, mannite, sucrose and salicin with production of acid and gas. GL M H S S
2. Indole-positive, methyl red (MR) positive, Voges-Proskauer (VP) negative, and citrate-negative (IMVC ++--).

* Indole + ve
 Tryptophan → produce indole

4 Lactose fermenter	4 Lactose non fermenter
* E. coli * Klebsiella * enterobacter	* salmonella * shigella * yersinia * proteus
* Citrobacter	

Antigenic structure

(O)
(H)
(K)

Serological classification system of E. coli strains is based on O (somatic) antigen of the cell wall lipopolysaccharide (LPS) and H (flagellar) antigen. Capsulated strains possess capsular (K) antigen.

Virulence factors

فactors of E coli →

A. Diarrhoeagenic E. coli have:

1. Pili (colonization factor).
2. Enterotoxins: Two types, heat-labile (LT) and heat-stable (ST), are produced by enterotoxigenic E. coli (ETEC) strains.
3. The shiga toxins: are produced by the enterohaemorrhagic E. coli (EHEC) strains.

B. Uropathogenic strains of E. coli, have fimbrial adhesins, exotoxins (haemolysins) and K antigen

C. The LPS causes endotoxic shock when released into the circulation.
 Lipo poly saccharide

endotoxin only for Gr - ve
 exotoxin mainly Gr + ve & Gr - ve not only for Gr - ve

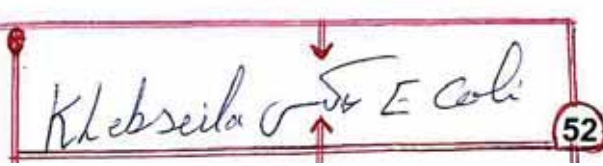
Diseases Caused by E. coli

1. Urinary tract infection (UTI)

UTI 80%
 لو حرقا

* Virulence factors
 Uropathogenic

- a- Community-acquired UTI:** E. coli is the commonest cause and accounts for >80% of infections. The uropathogenic strains of E. coli are present in the faeces and subsequently colonize the vagina and periurethral region. These organisms ascend into urethra (urethritis), bladder (cystitis), ureters, renal pelvis (pyelitis) and renal parenchyma (pyelonephritis).
- b- Hospital-acquired UTI:** It is usually associated with urinary catheters and caused by multi-resistant strains.



فactors of E coli →
 من المستفاد ان غالباً يكون M H S S

① E. coli & streptococcus & diphtheria & Klebsiella Cause Neonatal Meningitis

2. Neonatal meningitis: E. coli K1 is a common cause of neonatal meningitis.

→ Capsular antigen

3. Pneumonia, sepsis, septicaemia, and endotoxic shock may follow any of the E. coli infections particularly in neonates.

4. Diarrhoea: Diarrhoeagenic E. coli include the following types:

a. **Enterotoxigenic E. coli (ETEC):** The LT consists of 2 subunits A and B. It attaches via its B subunit to the gut mucosa, then the A subunit enters the cell and activates adenylate cyclase resulting in conversion of ATP to cAMP. Increased level of cAMP induces the active secretion of Cl^- and inhibits the absorption of Na^+ creating an electrolyte imbalance and loss of copious amounts of fluids from the intestine. LT has a mode of action similar to cholera toxin.

The (ST) stimulates the activity of guanylate cyclase in intestinal epithelial cells leading to formation of cGMP, resulting also in loss of fluids from the intestine.

Enterotoxigenic E. coli (ETEC) strains cause severe diarrhoea in infants and children and (traveler's diarrhoea) in adults. Infection is acquired through ingestion of contaminated food and water and can occur in an epidemic form.

b. **Enterohaemorrhagic E. coli (EHEC):** also known as verotoxigenic E. coli or

② shiga toxin-producing E. coli. Owing to production of shiga toxin, EHEC strains cause bloody diarrhoea or haemorrhagic colitis (a disease similar to shigella dysentery) and also haemolytic uraemic syndrome (HUS) as a potentially fatal complication. E. coli serotype O157:H7 is the most common strain associated with this disease. Ground beef (hamburger) has caused many outbreaks.

c. **Enteropathogenic E. coli (EPEC):** EPEC strains adhere tightly to the intestinal mucosa and interfere with water absorption by mucosal cells. They are a common cause of infantile diarrhoea.

d. **Enteroinvasive E. coli (EIEC):** EIEC strains cause a disease identical to that caused by Shigella spp. but do not produce shiga toxin.

e. **Enteraggagative E. coli (EaggEC)** adheres to intestinal mucosa in patches and produce heat-stable enterotoxin, causing persistent diarrhoea in children.

* Bacteria has toxins with AB struct. model

Laboratory diagnosis

A. Specimens include faeces and extraintestinal specimens such as urine, wound swabs, respiratory secretions, blood and CSF.

B. Direct detection

- 1- Gram-stained smear tells only that Gram-negative bacilli are present. It is useful only in specimens normally devoid of flora, e.g. CSF. → N. mening * تذكرة
- 2- E. coli K1 antigen is detected by agglutination in CSF in neonatal meningitis.

C. Cultivation

- 1- Specimens other than the blood and urine should be plated directly onto MacConkey agar as well as blood agar and incubated at 37°C.
- 2- Blood samples should be cultivated by the blood culture technique in cases of septicaemia and meningitis. Subcultures are plated on MacConkey agar and blood agar and incubated as above.
- 3- Urine should be quantitatively cultured to determine bacteruria (see Vol III).

D. Identification

- 1- After 24h incubation, colonies should be examined for morphology, Gram stain and oxidase.
- 2- Colony showing Gram-negative bacilli and is oxidase negative should be considered an Enterobacteriaceae organism.
- 3- E. coli usually produces rose pink colonies on MacConkey agar.
- 4- Colonies should be tested biochemically (see above).

E. Serological tests

E. coli from faeces should be further tested serologically and for virulence to confirm its enteric pathogenicity as follows:

1. Slide agglutination using specific antisera.
2. Tissue culture methods for toxin production (ETEC, EHEC), invasiveness (EIEC), and adherence (EPEC, EaggEC).
3. ELISA can be used also to test for toxin production.
4. DNA probe or PCR to detect genes of toxin production.

Antimicrobial susceptibilities

Antibiotic therapy of extra-intestinal E. coli infections should be guided by in vitro susceptibility testing because of the emergence of resistant strains.

In cases of diarrhoea, treatment depends on correction of dehydration and electrolyte imbalance. Selected antibiotics can be helpful.

N.B.: E. coli is used as an indicator of faecal pollution of water. Drinking water showing E. coli colony count above 4/dl is unacceptable for human use. Other organisms used to indicate faecal pollution of water are Enterococcus faecalis and Clostridium perfringens.

Klebsiella

capsulated

non motile

Lactose fermenter
G-ve Bacilli
all sugar ferment + gas + acid

IMVC --++

Klebsiella organisms are Gram-negative bacilli that are widespread in nature. *Klebsiellae* occur in two common habitats (1) the environment: in surface water, sewage, soil and on plants, and (2) mucosal surfaces of intestinal and respiratory tracts. Multi-drug resistant *Klebsiella* strains may prevail in the hospital environment and cause serious nosocomial infections. The genus includes *K. pneumoniae*, *K. ozaenae*, *K. rhinoscleromatis* and *K. oxytoca*. *K. pneumoniae* is the medically most important species.

Morphology

Klebsiella organisms are non-motile and usually capsulated Gram-negative bacilli. The capsule is the most important virulence factor. Because of this capsule, *K. pneumoniae* is highly virulent to mice.

Culture

Klebsiella species grow on MacConkey agar producing rose pink colony due to lactose fermentation, the colony is usually mucoid.

Biochemical reactions

Klebsiella ferments glucose, lactose, maltose, mannite, sucrose and salicin with production of acid and gas. IMVC of *K. pneumoniae* is --++.

Diseases

The following diseases are either community- or hospital-acquired and caused mainly by *K. pneumoniae* and much less frequently by *K. oxytoca*:

1. Urinary tract infection: It is the most common infection.
2. Pneumonia.
3. Wound and bloodstream infections with focal lesions (e.g. liver or lung abscess).
4. Neonatal sepsis: Septicaemia and meningitis in newborns and premature infants.

Klebsiella ozaenae is associated with atrophic rhinitis (ozena): a fetid, progressive atrophy of nasal mucosa. *Klebsiella rhinoscleromatis* is also associated with rhinoscleroma: a destructive granuloma of the nose and pharynx.

Antimicrobial susceptibilities

Emerging antimicrobial resistance among *Klebsiella* spp. is increasing. *K. pneumoniae* is intrinsically resistant to ampicillin. Resistance to cephalosporins is mediated β -lactamases. Therefore, routine *in vitro* susceptibility testing is required.

G.R. importance of Resistance susceptibility test in
to prevent intrinsic resistance

E. coli *motile* *Klebsiella* *motile*

Citrobacter, Enterobacter and Serratia → test efficiency and indicator of bacterial filters

Organisms of these genera are motile Gram-negative bacilli, typically found in soil, water and occasionally in the human respiratory and intestinal tracts and animal intestine. They cause opportunistic infections in humans similar to those caused by *K. pneumoniae*.

***Citrobacter**: It is similar to *E. coli* except in being citrate-positive.

***Enterobacter**: It is similar to *Klebsiella* except in being motile.

***Serratia**: Some strains produce red non-diffusible pigment and are used for testing efficiency of bacterial filters.

Salmonella

enterica
species
 bongori

Lactose non fermenter

Salmonellae are widespread in nature. They are commonly found in the intestinal tract of mammals, birds and reptiles. Poultry continues to be one of the primary reservoirs of the organism. Any raw poultry product, particularly eggs, should be assumed to contain salmonellae and, therefore, must be handled appropriately. In recent years, there have been numerous outbreaks of gastroenteritis (food poisoning) attributed to salmonella-contaminated eggs. Typhoid fever, which is endemic in Egypt, is caused by *Salmonella Typhi*.

Classification

Recently, the *Salmonella* genus is classified into two species based on biochemical reactions and DNA hybridization studies. The most important species is *Salmonella enterica*, which is further classified into six subspecies. The most important subspecies is the subsp. *enterica* whose strains are isolated from humans and warm-blooded animals.

More important is the serological classification of the genus *Salmonella* into serogroups and serotypes (see below). Currently, there are about 2435 *Salmonella* serotypes, out of them 1435 serotypes belong to the subsp. *enterica*. Although any

**Salmonella* serotype can cause infection, *Salmonella Typhi*, *S. Paratyphi A*, *S. Paratyphi B*, *S. Paratyphi C*, *S. Enteritidis* and *S. Typhimurium* are reported to be the most medically important serotypes.

Morphology

Salmonellae are Gram-negative bacilli and almost all are motile.

Culture

Salmonella grows as colourless colony on MacConkey and DCA media, after incubation at 37°C for 24 hours.

Biochemical reactions

The usual biochemical reactions include:

1. Fermentation of glucose, maltose and mannite with production of acid and gas. *ferment only 3 sugars*
2. *Salmonella Typhi* produces acid only. *glucose maltose mannite* → *produce gas Paratyphi*
Urease-negative and indole-negative. *not produce gas Typhi*
3. They usually produce H_2S .

Antigenic structure

The antigens used to define groups and types of *Salmonellae* include:

1. The O (somatic) antigens: heat-stable polysaccharides that form part of the cell wall lipopolysaccharide (LPS).
2. The H antigens: heat-labile proteins (flagellin) of the flagella that, in most of salmonellae, have 2 phases: phase 1 which is specific and phase 2 which is non-specific. *specific phase 1*
3. The Vi antigen: a heat-labile capsular polysaccharide antigen. *Vi antigen*

The genus *Salmonella* is subdivided into **O serogroups** according to the O antigen. The O antigen is composed of many antigenic factors; the major (dominant) factor will determine the group and will not be shared with other groups. The minor O factors can be shared with other groups. Therefore, **cross reactions** occur between these groups during serologic testing. Within the groups, **serotypes** are classified according to H and Vi (if present) antigens. *not in all serotypes*

Diseases Caused by Salmonella

1. **Salmonella food poisoning** (gastroenteritis or enterocolitis). It is worldwide infection caused by nontyphoidal *Salmonella* strains, commonly *S. Enteritidis* and *S. Typhimurium*. It is the most common salmonella infection. Disease transmission is usually linked to food of animal and poultry origins. Water or food contaminated with rat excreta can also transmit the infection. All ages are affected; the incidence is highest in infants. The disease is due to an intestinal infection accompanied by severe diarrhoea, fever, and abdominal cramps. The incubation period is 8-48 hours, and the disease lasts for 1 week or longer. The organism characteristically invades and replicates in the epithelial cells of small and large intestines (not in macrophages) leading to intestinal lesions and diarrhoea. Blood culture is usually negative but stools culture is positive up to several weeks. **Treatment** depends on correction of dehydration and electrolyte imbalance in addition to an antibiotic. *rat excreta contaminated*

2. **Typhoid fever** caused by *Salmonella Typhi*. A similar syndrome is caused by "paratyphoid" strains of *Salmonella*: *Salmonella Paratyphi A*, *Paratyphi B*, and *Paratyphi C*.

* *Staphylococcus aureus*
* *Clostridium perfringens* (enterotoxin) * *Bacillus cereus* emetic & diarrhoeal

osteomyelitis
pneumonia
meningitis



3. **Septicaemia (or bacteraemia)** represents about 5-10% of salmonella infections. The condition usually occurs in immunocompromised individuals.

After ingestion of salmonellae, they invade the intestinal mucosa and invade the bloodstream early without intestinal lesions. Bacteraemia results in metastatic abscesses and commonly manifest as osteomyelitis, arthritis, pneumonia and meningitis. The condition is commonly associated with **Salmonella** *Choleraesuis* and blood culture is usually positive particularly during the high fever.

Salmonella

Salmonella
② food poisoning
S. enteritidis
S. typhimurium
S. typhi fever
S. Paratyphi a, b, c
no gas = Typhi
has gas = ParaTyphi
sept bact
S. cholerae suis

Typhoid and Paratyphoid Fevers

The term **enteric fever** which includes both typhoid and paratyphoid, has been defined as 'a generalized infection of the reticuloendothelial system and intestinal lymphoid tissue accompanied by sustained fever and bacteraemia'. The typhoid tends to be severe, and paratyphoid to be milder. Globally, typhoid is far more common than paratyphoid.

Pathogenesis

Common Typhoid fever > Paratyphoid fever less common
② *S. Typhi* * *S. Paratyphi A, B, C*
③ Severe > less severe

Humans are the only reservoir, either patients or healthy carriers. The infection is transmitted through faecally contaminated food and water or person-to-person contact. The organism adheres to the mucosa of the small intestine, then invades to the submucosal layer. **Invasion of macrophages**, in the lymphoid tissues, and replication of the organism then take place. Salmonellae are then transported to lymphatics and mesenteric lymph glands. It disseminates to the bloodstream and a phase of bacteraemia (first week of illness) occurs. At the same time, uptake of the organisms by spleen and liver takes place where **replication of salmonellae inside macrophages** occurs for a period of time. The organism is then transported to the gall bladder to be excreted with bile into the intestine. **Re-invasion** of small intestine and uptake in Peyer's patches take place. The organism is excreted with faeces at the same time (second week of the disease). In about 25% of cases, the organism is excreted in the urine.

The incubation period is usually 10-14 days and the onset, with fever, malaise and headache, is slow, insidious and vague. During the first week, the fever becomes steadily higher on a stepwise pattern. During the second week, the fever may be steady or may swing.

Complications of enteric fever include relapse, perforation of the bowel, haemorrhage from the bowel ulceration, and carrier state.

Carrier state may last for 3 months or may persist to become chronic.

carrier
in bladder
gall bladder
urinary bladder

Laboratory diagnosis of enteric fever

Definitive diagnosis is only accomplished by isolation of salmonella from clinical specimens.

A. Specimens for culture are collected in the following ways:

- 1- Blood from the first day of illness. (1st week) of enteric fever
- 2- Faeces from the second week on. (2nd week) of enteric fever
- 3- Urine after the second week especially from those with urinary schistosomiasis. 25% of cases
- 4- Bone marrow is rarely indicated.

B. Direct detection by microscopy is useless.

C. Cultivation

- 1- **Blood or bone marrow culture:** Samples should be cultivated by the blood BCT culture technique. Subcultures are plated on MacConkey agar and incubated at 37°C.
- 2- **Stools culture:** Faecal specimens should be primarily inoculated directly on both solid media and enrichment (tetrathionate or selenite) broth. Differential selective solid media such as MacConkey, DCA and Salmonella-Shigella (SS) agar are used. Subcultures from enrichment broth are done after overnight incubation. All cultures are incubated at 37°C.

D. Identification

- 1- After 24h incubation, colonies should be examined for morphology, Gram stain and oxidase.
- 2- Colony showing Gram-negative bacilli and is oxidase negative is considered an Enterobacteriaceae.
- 3- Salmonella produces colourless (lactose nonfermenting) colonies on MacConkey agar and DCA.
- 4- Salmonella is identified by testing the colony for biochemical reactions (see before) or by slide agglutination using O serogroup antibodies.

E. Serodiagnosis of enteric fever by Widal test

If properly performed and carefully interpreted, Widal test would be diagnostic.

Widal test measures agglutinating antibodies to the O and H antigens of Salmonella Typhi and Paratyphi A and B.

It is a tube dilution agglutination test done by mixing serial (twofold) dilutions of the patient's serum with O and H antigens from representative salmonellae.

- The antibody titre is taken as the highest serum dilution showing agglutination.
- The results are reported by giving the titre for both O and H antibodies, and Vi antibody if included.
- Antibodies can be detected at the beginning of the second week onwards. At least 2 serum samples should be obtained at intervals of 7-10 days, to detect rising of antibody titre.

To avoid in endemic areas

IGM = recent infection

2011
* give account on 1st week of illness diagnosis
* give acc. on 2nd week

* 1st week in blood bec. bacteremia
* 2nd week faeces 25% urine

Blood → can't be stained
urine →
faeces → has many flora

solid media
&
enrichment

glucose
+ 3 → maltose
→ mannitol
+ acid
↑ gas
S. Para Typhi
S. Typhi
→ ve weak
→ ve indole
* TS I product
+ ve H₂S

Serodiagnosis after 2nd week

Serology

±

appear early and disappear fast
 appear = recent infect.
 O = LPS (Lipopolysaccharide) → Thymus independent

- In the antibody response of enteric fever, O antibody disappears faster than H antibody, and signifies active infection. On the other hand, H antibody signifies the type of infecting organism.

Results are interpreted as follows:

- High titre of O and H ($\geq 1:160$) or rising titre (fourfold or greater) suggests active infection.
- High titre of H alone ($\geq 1:160$) indicates past immunization or past infection.
- High titre of antibody to Vi antigen occurs in some carriers.

G.R.

Widal test not good diagnostic

Results of Widal test should be cautiously interpreted due to the following factors:

- The possible presence of cross-reacting antibodies, e.g. infection with other related members of *Enterobacteriaceae* and autoimmune diseases.
- Endemicity of the disease: Healthy people have antibody titre due to subclinical infection. Titres up to 1:40 are considered normal.
- Patients receiving early treatment show low titres.
- Vaccinated people have both O and H antibodies if recently immunized and H antibody alone in cases of past vaccination.

Prevention

A. Hygienic measures

- Avoid contamination of food and water by rodents or other animals that excrete salmonellae.
- Proper sewage treatment and chlorinated water supply.
- Pasteurization of milk and proper cooking of poultry, meat and eggs.
- Hand wash prior to food handling.
- Food handlers are examined to diagnose carriers, who should be treated or excluded from handling food.

B. Immunological measures

- TAB vaccine: A heat killed vaccine prepared by killing the organisms at 60°C for 30 min with 0.5% phenol added as a preservative. The suspension contains Salmonella Typhi, S. Paratyphi A and S. Paratyphi B. The vaccine is given as 2 S.C. doses 0.5 ml and 1 ml with one month interval, then a booster dose some months later.
- Acetone-killed bacterial suspension of Salmonella Typhi: It is given as 2 S.C. doses followed by a booster dose some months later.
- Oral typhoid vaccine: A live avirulent mutant strain of S. Typhi.
- The Vi capsular polysaccharide of S. Typhi. It is given intramuscularly.

① TAB = (S.T + S.PTA + SPTB) S.C.
 ② Acetone killed bact. = S.T heat killed vaccine
 ③ Oral Typhoid vaccine = S.T S.C.
 ④ Vi capsular polysaccharide I.M.

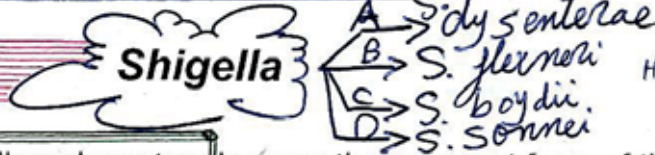
② S.C. vaccines
 @ oral
 @ I.M.
 enumerate

Vaccines S.C. → to delay abs. + prolong duration + potentiate

Antimicrobial susceptibilities

Multidrug-resistance in *S. Typhi* strains has become a problem. Because of the emergence of resistance to chloramphenicol, now the drug of choice for enteric fever and septicaemia is either ceftriaxone or ciprofloxacin. Ampicillin or ciprofloxacin should be used in chronic carriers of *S. Typhi*.

Freely you have received, freely give.
 * drug of choice $\rightarrow$ ceftriaxone
 * in chronicity $\rightarrow$ Ampicillin
 $\rightarrow$ ciprofloxacin



Shigella species cause bacillary dysentery in man; the severest form of the disease is caused by *S. dysenteriae* type 1.

Severest form of dysentery caused by *S. dysenteriae* type 1 due to shiga toxin

Morphology

Shigellae are nonmotile non-capsulated Gram-negative bacilli.

القالب + 0 حركه

Culture

Shigellae grow as colourless colonies on MacConkey and desoxycholate citrate agar (DCA) media, after incubation at 37°C for 24 hours.

Lactose non fermenter

facultative anaerobe

Biochemical reactions

- As a member of *Enterobacteriaceae*, all shigellae ferment glucose.
- All shigellae are lactose nonfermenters except *S. sonnei* which is a late lactose fermenter (fermentation is observed after 48 hours).
- All shigellae are mannite-fermenters except *S. dysenteriae*.
- Shigellae produce acid but no gas from fermentable sugars.
- All shigellae are urease and H₂S negative.

McQ

Antigenic structure

O antigen only

Shigellae are nonmotile and do not have H antigens. Serological typing is based on O antigens which identify both serogroups and serotypes of *Shigella*.

Classification of shigellae is based upon biochemical reactions and structure of the O antigen. There are four serogroups that are considered species (Table 7).

No gas
 Typhi, Flexneri, boydii, sonnei
 Salmonella, Shigella

Table (7): Serogroups, species and serotypes of *Shigella*

O Serogroups	Species	No. of O serotypes (serovars)	Useful sugar fermentations		
			Glucose	Lactose	Mannite
A	<i>S. dysenteriae</i>	13	+	-	-
B	<i>S. flexneri</i>	8	+	-	+
C	<i>S. boydii</i>	18	+	-	+
D	<i>S. sonnei</i>	1	+	Late	+

دysentery

Diagnosis $\rightarrow$ تقييد الجود

TST

Virulence factors

A. Invasiveness: The essential pathogenic process of bacillary dysentery is invasion of mucosal epithelium of the terminal ileum and large intestine where the organism is able to grow. The organism does not invade into the blood.

B. Shiga toxin: Shiga toxin is an exotoxin produced by *S. dysenteriae* type 1. The toxin can act as an enterotoxin and a neurotoxin that may cause meningismus and coma. In shigellosis, the toxin may damage blood vessels that could contribute to mucosal damage and may cause renal failure seen in haemolytic uraemic syndrome (HUS).

Pathogenesis of bacillary dysentery

Man is the only reservoir for shigellosis. It is characterized by a type of diarrhoea in which the stools contains blood and mucus. It is associated with heavy inflammation of the colonic mucosa and pseudomembrane formation.

Ingestion of few organisms (100-200 organisms) is able to cause disease, so person-to-person transmission can occur. Infections with *Shigella* spp. are often acquired by eating food washed by contaminated water or by drinking water contaminated with human faeces.

Laboratory diagnosis of bacillary dysentery

A. Specimens: Fresh stools.

B. Direct detection: Direct microscopy can not identify *Shigella* but is done to differentiate bacillary from amoebic dysentery. In bacillary dysentery, large number of PMNLs and some erythrocytes are seen under the microscope.

C. Cultivation: Faecal specimens should be initially inoculated onto MacConkey and DCA as well as enrichment broth (selenite broth). Subcultures from enrichment broth are done. All cultures are incubated at 37°C.

D. Identification

- After 24h incubation, colonies should be examined for morphology, motility, Gram stain and oxidase.
- Colony showing nonmotile Gram-negative bacilli and is oxidase negative is considered an Enterobacteriaceae.
- Shigella* produces colourless (lactose nonfermenting) colonies on MacConkey agar.
- Shigella* is identified by testing the colony for biochemical reactions or by slide agglutination using O serogroup antibodies (see table 7).

Prevention and control

Detection of *shigella* carriers and public health measures are recommended for control of shigellosis.

Salmonella Hygienic measure

No Specific prevention of *Shigella*

Urease → *Proteus* (free) you have received; freely give.
 → Stomach (*Helicobacter*)

Antimicrobial susceptibilities:

Susceptibility testing should be done because of the widespread antimicrobial resistance among *Shigella* strains. The following antimicrobial agents are recommended for treatment of *Shigella* infection: ampicillin, fluoroquinolones (e.g. ciprofloxacin), nalidixic acid, and trimethoprim/sulfamethoxazole.

① *Proteus*, ② *Providencia* and ③ *Morganella*

These are 3 genera of motile Gram-negative bacilli, usually pleomorphic. They have a number of diagnostic features in common and therefore are studied together. These organisms occur in the environment as well as in human and animal intestines. Important species include *Pr. mirabilis*, *Pr. vulgaris*, *Providencia rettgeri* and *M. morganii*. These species are lactose nonfermenter (LNF), phenylalanine deaminase-positive and urease-positive. *Proteus* swarms on nutrient agar but *Providencia* and *Morganella* do not. Since *Proteus* is LNF and H_2S positive it may be confused with *Salmonella* while identifying such pathogenic isolates from stools cultures.

Pathogenicity: *Proteus*

- Infections are usually nosocomial, including:
1. Urinary tract infection especially caused by *Pr. mirabilis*.
 2. Wound infections and abscess formation.
 3. Respiratory infections: Otitis media and pneumonia.
 4. Septicaemia and meningitis.

M. morganii causes UTI, wound infections and diarrhoea.

Providencia rettgeri causes urinary tract, wound and burn infections.

Treatment: Antibiotic susceptibility testing should be performed because of the high frequency of resistance to antibiotics. Newer cephalosporins or quinolones are usually effective.

Diagnosis = Culture + Bioch + Identification
Yersinia

Members of this genus are Gram-negative, facultative anaerobic rods that are smaller than other members of *Enterobacteriaceae* and stain in a bipolar fashion. There are 3 important human pathogenic species: *Y. pestis*, the aetiologic agent of plague, and the enteropathogenic species, *Y. pseudotuberculosis* and *Y. enterocolitica*.

Yersinia enterocolitica and Y. pseudotuberculosis

Y. enterocolitica and *Y. pseudotuberculosis* cause enteric disease due to contaminated food or water. *Y. enterocolitica* is widely distributed in nature in aquatic and animal reservoirs, with swine serving as a major reservoir. Refrigerated foods are potential vehicles because *Y. enterocolitica* can grow at refrigeration temperatures. Incriminated foods include meat (pork, beef, lamb), fish, and raw milk. *Y. enterocolitica* is not part of the normal human flora. Disease (yersiniosis) can range from mild diarrhoea to what appears to be acute appendicitis to a typhoid-like septicaemia.

Yersinia pestis

Plague bacillus has historically been a deadly pathogen, inflicting Europeans in epidemics during the fourteenth, fifteenth and sixteenth centuries killing tens of millions. Plague is a zoonotic disease primarily affecting rodents which act as reservoirs.

Morphology

Y. pestis as well as other members of the genus *Yersinia* are Gram-negative coccobacilli that have characteristic bipolar staining, producing a "closed safety pin" appearance. While there is no true capsule, a carbohydrate-protein envelope, termed capsular antigen or fraction 1 (F1), is formed during growth above 33°C. The organism is nonmotile and non-spore forming.

Cultural characteristics

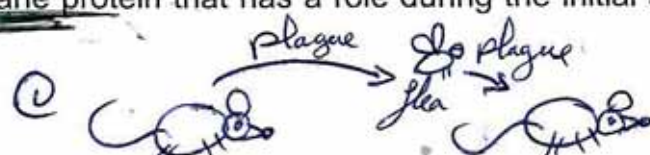
Y. pestis can be cultured on blood agar and MacConkey agar. *Y. pestis* forms small pinpoint colourless (lactose nonfermenting) colonies after 24 h incubation at 25°C.

Antigens and virulence factors

- Fraction 1 capsule:** The F1 protein forms a large gel-like capsule.
- Murine toxin:** A toxic protein for mice and rats.
- Catalase:** resist intracellular killing
- Invasin:** An outer membrane protein that has a role during the initial stages of infection.

Epidemiology

Transmission of plague between rodents is accomplished by fleas. The rat flea (*Xenopsylla cheopis*) acquires *Y. pestis* from an infected blood-meal. Transmission to man can occur by one of the following ways:





1. Flea-borne, from infected rodents through the bite of the flea.
2. Direct contact with infected tissues or fluids from handling sick or dead animals.
3. Respiratory droplets from cats and humans with pneumonic plague.

In bioterrorism, the organism may be delivered by aerosol to cause pneumonic plague, or using infected fleas to cause bubonic plague.

Anthrax
Bioterrorism
But with force

Clinical features

- Bubonic plague: Enlarged, tender lymph nodes, fever, chills and prostration.
- Septicaemic plague: Fever, chills, prostration, abdominal pain, shock and bleeding into skin and other organs.
- Pneumonic plague: Fever, chills, cough and difficulty in breathing, shock and death if not treated early.



Laboratory diagnosis

A. Specimens: Blood, sputum or lymph node aspirates.

B. Direct detection

- 1- Gram or methylene blue stain to see the characteristic bipolar staining of coccobacilli.
- 2- A positive fluorescent-antibody test.
- 3- Detection of Y. pestis DNA by PCR in blood, sputum, CSF or bubo aspirates.

C. Cultivation and Identification

Specimens are inoculated on blood agar. Y. pestis takes 2 days to yield visible colonies. The colonies are identified as follows:

- 1- Fraction 1 (F1) antigen or capsular antigen detected by FA tests.
- 2- Plaque bacilli are susceptible to lysis by a specific bacteriophage.
- 3- Biochemical tests.

D. Animal inoculation of the clinical specimen into white rat or guinea pig. The animal may die within 2-5 days and the organism can be detected.

Curved = $\hookrightarrow$ cjr
no capsule =
motile = 0 cjr

Gram -ve 18 - 11

Chapter 11

VIBRIO

* Gram -ve
* 0 = motile Bacilli
(single polar flagellum)
* Aerobic

Characteristic features:

Members of the genus *Vibrio* are:

1. Highly motile, Gram-negative, curved bacilli with a single polar flagellum.
2. Facultative anaerobes.
3. Tolerant to alkaline conditions but destroyed by low pH = destroyed by acids
4. Oxidase-positive.
5. String-positive.
6. Ferment sugars without gas production and reduce nitrates.
except lactose

Gr. why they are not enterobact bec. oxidase +ve string +ve

Vibrios are one of the most common organisms in both sea and freshwater habitats and in association with aquatic animals. *Vibrio cholerae* is the most clinically important species. Other important species are *V. parahaemolyticus* and *V. vulnificus*. The flagellar (H) antigens are shared with all vibrios and, therefore, are of no use in distinguishing strains. On the other hand, O antigens are specific and can distinguish species into serogroups and serotypes.

String test:

* What is string test? what it is used for?

The string test is useful for ruling out bacteria that share similarities to vibrio species, particularly *Aeromonas* species. The test is done by emulsifying a large colony in a small drop of 0.5% sodium desoxycholate in sterile distilled H₂O. Within 60 sec the cells lyse (loss of turbidity) and DNA strings when a loopful is lifted (up to 2 to 3 cm) from the slide. All *V. cholerae* strains as well as most other vibrios are positive, whereas *Aeromonas* strains are negative.

Vibrio cholerae

→ faeco oral infection

Vibrio cholerae serogroups O1 and O139 are the causative agent of epidemic cholera. On the other hand, *V. cholerae* non-O1/O139 are involved in sporadic forms of cholera-like diarrhoeal disease, but not in epidemics. Also, some non-O1/O139 strains are invasive and cause septic infections.

Morphology (mentioned above)

Cultural characters

V. cholerae is facultative anaerobe and grows best under alkaline conditions. The cholera vibrios can be grown on alkaline peptone water (pH 8.5), nutrient agar at slightly alkaline pH and the selective indicator medium thiosulphate-citrate-bile salts-sucrose (TCBS) agar. On alkaline peptone water, a surface pellicle, containing the organism, is formed after an incubation period of 6 to 8 h at 37°C. *Vibrio cholerae* produces yellow colonies on TCBS owing to sucrose fermentation.

yellow colonies

MCQ

Biochemical reactions

Vibrio cholerae strains ferment glucose, maltose, mannite and sucrose but **not** lactose, with production of acid but not gas. *V. cholerae*, like other vibrios, reduces nitrate and is oxidase-positive. It is indole-positive.

TSI

Serological characters and biotypes of *V. cholerae*

O antigens distinguish strains of *V. cholerae* into serogroups designated by numerals. The serogroup O1 has three distinct **serotypes**, named **Ogawa**, **Inaba** and **Hikojima**. Each serotype may display the "classical" or **EI Tor** biotype.

O₁ serotypes
① Ogawa
② Inaba
③ Hikojima

classical EI Tor serotype Biotypes

Cholera

→ Patient dies from dehydration

Cholera is a severe diarrhoeal disease induced by cholera enterotoxin secreted by *V. cholerae* serogroups O1 and O139.

virulence factor

Pathogenesis

The natural reservoir of the organism is humans, but may be the aquatic environment. Infection is transmitted primarily by the faeco-oral route through contaminated water or food. Direct person-to-person spread is not common because the infectivity dose is high (10^7 - 10^{12}). Vibrios are sensitive to acid, and most die in the stomach.

* because faeco oral inf where its sensitive to acid

* give reason high infective dose of
* give reason no person-personal cholera

If the bacteria pass the stomach, virulent organisms will penetrate the mucous layer of the small intestine and specifically adhere to its mucosa by fimbriae and other colonization factors. *V. cholerae* will then multiply and secrete the potent cholera enterotoxin (CT).

* virulence factor

Cholera toxin (or **choleragen**) contains **5 binding (B)** subunits and **an active (A)** subunit. The toxin binds through its B region to specific receptors on the intestinal epithelial cells and releases the enzymatically active (A) subunit that enters the cells and activates the adenylate cyclase enzyme causing a rise in cAMP production. This causes massive secretion of electrolytes (Na^+ , K^+ , Cl^- and HCO_3^-) and water into the lumen of the small intestine.

* because high dose infection because it's sensitive to acidity

Enterotoxigenic E. coli

Clinical Manifestations

Following an incubation period of **6 to 48 hours**, cholera begins with an abrupt onset of massive watery diarrhoea. Several litres of fluid may be secreted within hours, leading to hypovolaemic shock. The watery diarrhoea is speckled with flakes of mucus and epithelial cells "rice-water stools" and contains enormous numbers of vibrios. Vomiting usually occurs. The disease runs its course in **2 to 7 days**; the outcome depends upon the extent of water and electrolyte loss and the adequacy of treatment. Death may occur from hypovolaemic shock, metabolic acidosis and uraemia resulting from acute tubular necrosis.

rice water stools = case (lies)

① diphtheria < B
② Anthrax < 2A B₁

AB toxins



Diagnosis

The diagnosis is suggested by the characteristic clinical picture. Patients with severe secretory diarrhoea must receive fluid and electrolyte replacement regardless of aetiology. There are two approaches for the laboratory diagnosis of cholera:

A. In endemic areas or during spread of an epidemic

→ direct detect is enough

A wet mount of liquid stools is examined microscopically. The characteristic darting motility of vibrios is stopped by specific antibody. Another rapid method is the use of direct fluorescent antibody test on the stools smear.

B. The initial (primary) case of an epidemic in non-endemic areas:

Full bacteriologic diagnosis is essential to apply the required infection control measures.

1- Cultivation

of stools or rectal swab samples is primarily done on alkaline peptone water. A surface pellicle is formed after an incubation of 6 to 8 h at 37°C. Subcultures, from the surface pellicle, are done on TCBS and incubated overnight at 37°C. *V. cholerae* yields large yellow translucent colonies on TCBS.

2- Presumptive identification

The growth is identified as mentioned in table (8):

Table (8): Identification of *V. cholerae*.

Test	<i>V. cholerae</i> reactions
Gram stain	Small, Gram-negative curved rods
Wet mount	Shows rods with darting motility
Oxidase	Positive
String	Positive
Triple sugar iron agar	Acid yellow slant/ acid yellow butt, no gas produced

TSI

3- Confirmation

Microorganisms can be confirmed as cholera vibrios by a rapid slide agglutination test with specific antiserum. It should be noted that *V. cholerae* cultures that fail to agglutinate in either O1 or O139 antisera should be reported as non-O1/O139 *V. cholerae*.

4- Biochemical tests

Although demonstration of typical agglutination essentially confirms the diagnosis, biochemical tests are helpful (see above).

* all sugar ferment except Lactose with acid only
* oxidase +ve
* indole +ve

Treatment

Treatment of cholera consists essentially of replacing fluid and electrolytes either intravenously or orally.

Antibiotics such as tetracycline, to which the vibrios are generally sensitive, are useful adjuncts in treatment. Other antimicrobial agents that can be used include furazolidone, trimethoprim-sulfamethoxazole, chloramphenicol or ciprofloxacin.

Control

1. **Sanitation:** Application of sanitary principles that protect drinking water and food from contamination with human faeces.

2. **Development of a vaccine:** The development of an inexpensive, effective cholera vaccine that provides long term protection is not yet available. However, three vaccines are now commercially available:

- Cholera (koll's) vaccine: containing extract of killed bacteria. It is given intramuscularly.
- Whole cell/B subunit vaccine: containing killed bacteria and the B subunit of cholera toxin. It is given orally.
- Live attenuated vaccine: It is given orally.

Other vibrio infections

1. **Vibrio parahaemolyticus** It is a halophilic (salt-loving) vibrio associated with enteritis and is acquired by ingestion of raw or improperly cooked sea foods. Enteritis is self limited and manifests as a watery diarrhoea or as a dysenteric syndrome. The thermostable direct haemolysin or what is known as the Kanagawa haemolysin is the primary virulence factor of V. parahaemolyticus strains.

2. **V. vulnificus**: Another halophilic vibrio, which ferments lactose and for this reason was called the lactose positive vibrio. It has been associated with wound infections as well as fatal septicaemia.

3. **Non-O1/O139 serogroups of V. cholerae**: Mentioned before.

① Salty
② Fresh

Aeromonas

* -ve string
* non Cap2 * non curved
② * Gram -ve * Bacilli * motile

Aeromonas organisms are Gram-negative bacilli, motile with polar flagella, and otherwise similar to vibrios but string-negative. They are widespread inhabitant in salty, fresh, chlorinated and unchlorinated water supplies worldwide. **Aeromonas hydrophila** is the most important species. A. hydrophila infections are acquired through open wounds or by ingestion of food or water. A. hydrophila has frequently been found in fish and shellfish as well as in meat and poultry. Infections include:

- ① Bacteraemia and septicaemia in immunocompromised patients,
- ② Wound infections (cellulitis, myonecrosis, and ecthyma gangrenosum),
- ③ Gastroenteritis (a watery diarrhoea and a dysenteric illness).

Bacterial wound infection
gastroenteritis

Chapter 12
 @ Gram -ve = curved or (S) shaped
 @ darting motility < unipolar
 Bipolar unsheathed flag.

Microaerophilic
 = require ↑ by hydrogen for growth
 @ motile → grow at 15°C

CAMPYLOBACTER AND ARCOBACTER

③ Curved ④ motile ⑤ S shaped

@ curved ② S shaped

Campylobacter and Arcobacter are two closely related genera of curved, or S-shaped, slender (0.2 µm wide), nonspore-forming, Gram-negative rods.

A. **Campylobacter** species have the following features:

1- A single unsheathed flagellum either monopolar or bipolar and exhibit darting motility.

2- Microaerophilic and may require increased hydrogen for growth.

B. **Arcobacter** species have similar features but are able to grow at 15°C while Campylobacter species are not.

Campylobacter jejuni accounts for about 90% of the Campylobacter illnesses.

Other species that have been associated with human illness include *C. fetus* and *C. upsaliensis*.

Campylobacteriosis

① enteritis
 ② extra intestinal
 ③ guillain Barre syndrome
 = (acute paralytic disease)

Campylobacter infections or campylobacteriosis include the following forms:

1. **Campylobacter enteritis**: Campylobacter diarrhoea is as common as *Shigella* and *Salmonella*. The diarrhoea can be mild to severe, and watery or bloody.

2. **Extraintestinal infections**; such as reactive arthritis, bacteraemia, meningitis, pneumonia, abortion and acute cholecystitis.

3. **Guillain-Barre syndrome**: It is an acute paralytic disease of the peripheral nervous system. It is an autoimmune disease attributed to antibodies, against *C. jejuni*, that cross react with antigens on neurones.

Pathogenesis and clinical manifestations of campylobacter enteritis

Campylobacters are carried in the intestinal tract of a wide variety of birds and animals. They can establish a temporary carrier state in humans. Disease is most frequently associated with consumption of contaminated food and water. The foods include raw milk, meat, poultry, shellfish, fruits and vegetables.

Modes of transmission are faeco-oral, person-to-person, and rarely sexual contact (in homosexual men). Exposure to sick kittens has also been associated with outbreaks.



sexual transmission

oral sex = faeco-oral

C. jejuni adheres to epithelial cells or mucus, invades and destroys epithelial cells in the jejunum, ileum and colon. Some strains of *C. jejuni* produce an LT enterotoxin that causes watery diarrhoea and a cytotoxin that causes bloody diarrhoea.

Children under 5 years and young adults (15-29) are the most frequently affected age groups. The incubation period is 2-5 days. The spectrum of disease can range from asymptomatic to severe, life-threatening enteritis. However, most infections are self-limited and illness generally lasts 7-10 days. Bacteraemia is common, and *Campylobacter* is frequently isolated from the bloodstream.

asymptomatic
+
life threatening
or
severe
mostly
Self limited

Laboratory diagnosis

diarrhoeal = stool + rectal swab
systemic = Blood sample

A. Specimens: Stools and rectal swabs from patients with diarrhoea, and blood in cases of extraintestinal infections.

B. Direct detection

1- Gram-stained smears of stools may show typical comma or S-shaped rods.

2- Dark field or phase contrast microscopy may show typical darting motility in wet mounts.

GR. because it's 0.2 μ m wide slender

*motility not with gram But with wet mount

C. Cultivation

- Primary isolation on selective media in combination with a filtration method is the optimal method.
- Selective media include media with added antibiotics (e.g. Skirrow's medium). Cultures are incubated in microaerophilic atmosphere (5% oxygen and 10% CO₂). Incubation temperature is usually at 42°C since most species are thermophilic such as *C. jejuni*, but the mesophilic species *C. fetus* and *C. upsaliensis* grow best at 37°C for 2 days.

What is Skirrow's medium?

What is importance of filtration?

The filtration method is accomplished by using a filter with a pore size (0.25 to 0.45 μ m) sufficiently large to permit passage of the small campylobacters but small enough to exclude larger faecal organisms. The filtrate is then inoculated on a nonselective medium e.g. chocolate agar and incubated as mentioned above.

D. Identification: Growth of *C. jejuni* is identified biochemically.

Treatment

Replacement of fluids and electrolytes is the main line of therapy. Erythromycin is the antibiotic of choice. Ciprofloxacin and tetracycline are alternative choices; however, they should be avoided in young children.

Control

Proper cooking of chicken, pasteurizing milk and chlorinating drinking water will kill the bacteria.

Chapter 13

Campylobacter ٢٥/١٥

HELICOBACTER

gastric urease +ve

enterohepatic urease -ve

The genus *Helicobacter* includes spiral or curved, **microaerophilic** Gram-negative non-spore forming bacilli. All *Helicobacter* species are **oxidase-positive**. They are motile and usually have multiple bipolar sheathed flagella, but *Helicobacter pylori* has multiple **monopolar** sheathed flagella. *Helicobacter* species are included in two categories, **Gastric** and **Enterohepatic**. Gastric helicobacters are strongly **urease producers**; enterohepatic helicobacters are negative for urease. *H. pylori* is one of the gastric members, it is the most important helicobacter species since it is the causative agent of **peptic ulcer**. The latter is now approached as an infectious disease.

Enterohepatic *Helicobacter* species are linked with inflammation or malignant transformation in humans.

Helicobacter pylori

No sugar ferment

Morphology (mentioned above)

Culture

or chocolate

(Skirrow medium)

Fresh blood or serum agar media are used for culture of *H. pylori*. The organism requires **microaerophilic** condition (5% O₂ and 10% CO₂), high humidity and incubation at 37°C for 3-6 days. Growth of *H. pylori* neither occurs at 25°C nor at 42°C.

Biochemical reactions

All helicobacters are **oxidase positive** and do not ferment sugars. *H. pylori* produces large quantities of **urease**.

Epidemiology and transmission

@ faeco oral

@ person to person

H. pylori has been found in the stomach of humans in all parts of the world. There appears to be no reservoir of *H. pylori* aside from the human stomach. Direct **person-to-person transmission** of *H. pylori* usually occurs within families. **oral transmission** is perhaps the most important. *H. pylori* infection in adults is usually **chronic** and will not heal without specific therapy.

↓ infective dose

② faeco oral

① Gram - ve Bacilli non spore forming

② Curved + S shaped + spiral

③ motile ~~with~~ sheathed flagella

④ urease ~~+~~ +ve gastric

⑤ microaerophilic 5% O₂ / 10% CO₂ in H. pylori

⑥ not thermophilic 37°C no 25 42

⑦ high humid

⑧ slow growth 3-6 days

Virulence of *H. pylori* → VacA toxin → Cag PAI → Pathog. Island

Pathogenesis

- **Persistent colonization:** Urease production and motility are essential for colonization. Urease hydrolyzes urea into carbon dioxide and ammonia, the latter permits *H. pylori* to survive in an acidic environment.
- *H. pylori* strains may produce the **vacuolating cytotoxin VacA**. The toxin inserts itself into the epithelial cell membrane and forms a channel through which bicarbonate and organic anions can be released, providing the bacterium with nutrients. VacA also induces apoptosis.
- Pathogenic *H. pylori* strains contain the **cag pathogenicity island (cag-PAI)**. It is a chromosomal region encoding proteins involved in the induction of interleukin-8 production. Interleukin-8 is a chemokine which serves as a potent inflammatory mediator recruiting and activating neutrophils in the process of gastritis.

Clinical outcomes of *H. pylori* infection

Chronic gastritis will develop in virtually all persistently colonized people, but 80 to 90 % will never have symptoms. The clinical course depends on bacterial and host factors. Thus, **duodenal or gastric ulcers**, mucosal atrophy, **gastric carcinoma** or gastric lymphoma may follow the chronic gastritis.

Diagnosis

- A. Specimens:** at least two biopsies are taken from the pylorus during endoscopy.

No stool culture
only Biopsies
But Antigen assay
ELISA of stool
specimen test

B. Direct detection

- 1- **Histologic assessment:** *H. pylori* can be visualized with hematoxylin and eosin-stained sections or using special stains.
- 2- **Biopsy urease tests:** the gastric biopsy sample is placed onto the urea-containing media. The result can be obtained in 1 to 24 h

C. Cultivation

- 1- Fresh blood or serum agar media either selective (Skirrow's medium) or nonselective (chocolate agar) are inoculated with gastric biopsies.
- 2- Cultures are incubated in microaerophilic atmosphere, high humidity and at 37°C for 3-6 days.

D. Identification

- 1- *H. pylori* colonies appear pinpoint and translucent.
- 2- *H. pylori* is oxidase positive and strong urease producer.
- 3- It does not ferment sugars.
- 4- It can also be identified by DNA probes and PCR.



E. Serology

Infection of the gastric mucosa with *H. pylori* results in systemic as well as local immune responses, including production of specific IgG, IgM and IgA in serum and secretory IgA in the stomach. These antibodies are usually detected by ELISA tests.

F. Special tests

1- **Urea breath tests:** Urea is provided as a substrate which is ingested as either [^{13}C] or [^{14}C] urea. *H. pylori* urease hydrolyzes the ingested urea into labeled bicarbonate, which is exhaled as labeled CO_2 .

2- **Antigen assay for *H. pylori* in stools by ELISA:** This test performs well in children of all ages.

Treatment of *H. pylori* infection

The goal of *H. pylori* treatment is the complete eradication of the organism. Multidrug regimens are required to attain successful cure. The regimens should be a combination of antibiotics with "antacids". Either a macrolide (e.g. clarithromycin) or a nitroimidazole (e.g. metronidazole) must be included as one of the antibiotics used. Antacids are combined because gastric acidity influences the effectiveness of some antibiotics that are chosen in the regimen.

G.R. Administration of 2 antibiotics
↑ effect of antibiotic 1 antacid

Recommended triple therapy regimen is 1) a proton-pump-inhibitor (prilosec or prevacid) along with 2) clarithromycin and 3) amoxicillin or 1) bismuth salts along with 2) tetracycline and 3) metronidazole. Treatment should last two to four weeks.

* Triple therapy :-
2 Antibiotic
+
1 Antacid

NON-FERMENTATIVE GRAM-NEGATIVE BACILLI

Pseudomonas

Pseudomonas spp. are aerobic, non-spore-forming, motile, non-fermentative Gram-negative bacilli. Most species are oxidase positive and catalase positive. *P. aeruginosa* is the most important species.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is worldwide in distribution with a predilection for moist environments: in water and soil and on plants. It is a part of the normal microbial flora, particularly in the gastrointestinal tract and moist body sites. It is also found in the hospital environment especially in moist places.

Morphology: It has the same morphology as mentioned above.

Culture: *P. aeruginosa* grows on simple media. It usually produces diffusible exopigments, and has a grape-like odour. When the yellow pyoverdine pigment combines with the blue pyocyanin pigment, the bright green colour characteristic of *P. aeruginosa* colonies is created. It causes complete haemolysis on blood agar. Although optimal growth temperature is 37°C, it can grow at 42°C. It grows on MacConkey agar producing colourless colonies.

Biochemical activities: Oxidase-positive, indole-negative, carbohydrate non-fermenter; acid is produced from glucose only oxidatively.

Pathogenicity: *P. aeruginosa* is a significant human pathogen particularly in immunocompromised patients. It is a major cause of hospital acquired (nosocomial) infections. The organism is considered invasive and toxigenic and may cause pyogenic infections.

P. aeruginosa infections may be community-acquired or nosocomial:

A. Community-acquired *P. aeruginosa* infections:

- 1- Folliculitis. *skin infections*
- 2- External ear infections in swimmers (swimmer's ear), diabetics and the elderly.
- 3- Eye infections usually follow minor trauma to the cornea. These infections are frequently associated with contact lens use. Corneal ulcers may develop and lead to blindness if not promptly treated.



- 4- Osteomyelitis in children and in intravenous-drug users.
- 5- Endocarditis in intravenous-drug users.

B. Nosocomial *P. aeruginosa* infections:

- 1- Respiratory infections especially in intubated patient in intensive care units.
- 2- Urinary tract infections.
- 3- Wound infections and septicaemia.
- 4- Meningitis following lumbar puncture.
- 5- Chronic lung infection in cystic fibrosis.

Antimicrobial susceptibility and treatment

P. aeruginosa is resistant to many antibiotics. Therefore, *in vitro* testing is needed to give the appropriate antibiotics. Combined antibiotic treatment is usually indicated in serious infections. Prevention of nosocomial infections should be part of infection control procedures applied in the hospitals.

G.R. Importance?
because Multiple drug resistance

Acinetobacter

Acinetobacter species are oxidase-negative, non-fermentative, non-motile, strictly aerobic Gram-negative coccobacilli. They are free living saprophytes found in soil, water, foods and in hospitals. They can be found on inanimate surfaces and as commensals on the skin of humans and animals. In recent years, antibiotic resistant strains prevailed in hospitals and caused outbreaks of nosocomial infections, particularly in ICUs. It may cause pneumonia, urinary tract infection (UTI), meningitis, skin, and wound infections, that may progress to septicaemia. It can also cause community-acquired infections especially pneumonia and UTI.

MCQ
2/8

Chapter 15

HAEMOPHILUS

Ex 8 live 10:5
motility V.

* growth factors
X factor (Haematin)
V factor (NAD)

Members of the genus *Haemophilus* are facultative anaerobes, small, pleomorphic Gram-negative bacilli that require certain growth factors that are present in the blood; the genus name is derived from the Greek words meaning "blood-loving". These growth factors are **X-factor** (present in haemin or haematin) and **V-factor** (NAD). Some *Haemophilus* species require both factors while other species require only one of them for growth. Both factors are found within blood cells. Chocolate agar, which contains heated lysed blood, provides these factors and, therefore, is suitable for these organisms.

why *Haemophilus* cultured on chocolate agar?
because contain heated lysed growth factors

The medically important *Haemophilus* species are:

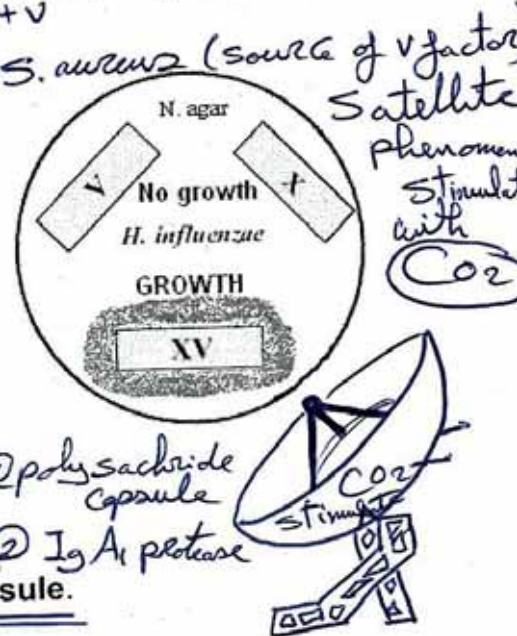
1. *H. influenzae*.
2. *H. ducreyi*.
3. *H. influenzae* biogroup *aegyptius* or *H. aegyptius* (Koch-Weeks bacillus).

* Gram -ve ~~Cocci~~ Coccobacilli
* Pleomorphic, filamentous
* Capsulated (polysaccharide) → 6 serotype → Hib = most important

H. influenzae is a slender, short Gram-negative rod or coccobacillus, that may be pleomorphic with many filamentous forms. Some strains are capsulated; the capsule is polysaccharide, antigenic and is important in pathogenicity. According to the polysaccharide capsule there are six serotypes a-f. *H. influenzae* type b (Hib) is the most important. Organisms occur as normal flora in the nasopharynx.

Cultural characteristics

H. influenzae requires both X-factor and V-factor for growth. Therefore, good growth is obtained on chocolate agar. It does not grow on blood agar unless provided with free V-factor from a source like *S. aureus* culture. Therefore, *H. influenzae* can grow on blood agar if *S. aureus* is grown at the same time on the medium. This condition is known as **satellitism**. Growth is stimulated by CO₂.



Virulence factors and clinical significance

1. The major virulence factor is the polysaccharide capsule.
2. IgA1 protease helps adherence to the mucosa.

Infection is transmitted from person-to-person by inhalation of respiratory droplets.

Mode of transmission

→ person to person
→ Inhalation

- ① *N. meningitidis*
 ② *strep pneumoniae*
 ③ *Haemophilus influenza*

G.R. at this age?
 bec. thymus
 independent
 antigen

A. Encapsulated types of *H. influenzae* particularly type b (Hib) cause:

- 1- Bacterial meningitis and epiglottitis in children less than 5 years of age.
- 2- Pericarditis, pneumonia, septic arthritis, osteomyelitis and facial cellulitis in the same age group.

These infections are usually accompanied by bacteraemia. It is observed that the recently used *H. influenzae* type b (Hib) vaccine reduced the incidence of these serious infections.

B. The nontypable (noncapsulated) strains cause

- 1- Pneumonia and bacteraemia in adults and older children, in presence of predisposing factors e.g. viral infections, malignancy and cystic fibrosis.
- 2- Otitis media and sinusitis (next to *S. pneumoniae*).

Laboratory diagnosis

A. Specimens include CSF, blood, pus and respiratory secretions

B. Direct detection in clinical specimens

- 1- Gram-stained smear: Gram-negative coccobacilli associated with PMNLs.
- 2- Quellung reaction. $\rightarrow$ for swelling caps
- 3- Antigen detection in CSF by latex agglutination test.
- 4- PCR.

C. Cultivation

- 1- Specimens other than the blood should be plated directly onto chocolate agar and incubated at 37°C with 5% CO_2 .
- 2- Blood samples should be cultivated by the blood culture technique. Subcultures are plated on chocolate agar and incubated as mentioned above.

D. Identification

An organism that grows only in the presence of both X- and V-factors is presumptively identified as *H. influenzae*. Definitive identification can be made by:

- ① Colony Morphology: $\left\{ \begin{array}{l} \text{caps large opaque} \\ \text{non caps small transparent} \end{array} \right.$
 - a- Noncapsulated strains produce small transparent flat colonies.
 - b- Capsulated strains produce larger opaque mucoid colonies.
- 2- Gram-stained smears: show Gram negative coccobacilli.
- 3- Detection of the capsular polysaccharide by Quellung reaction, latex agglutination or fluorescent-antibody staining.
- 4- DNA probes.

Treatment

Ampicillin and chloramphenicol that were used for treatment of Hib meningitis have been replaced by third generation cephalosporins. Cefotaxime or ceftriaxone is the antibiotic of choice.

Chapter 16

BORDETELLA

Bordetella pertussis

Has Vaccines
Killed whole Acellular

→ Whooping Cough

Bordetellae are small Gram-negative coccobacilli. They are strict aerobes.

B. pertussis is the most important species of the genus and causes the respiratory disease whooping cough (pertussis).

Virulence Factors and Pathogenesis

Pertussis pathogenesis occurs in two stages:

A- Colonization stage (about 10 days) during which the organism can be detected in large numbers in pharyngeal cultures, and the severity and duration of the disease can be reduced by antimicrobial treatment. The adherence mechanisms of *B. pertussis* involve the two most important colonization factors: filamentous haemagglutinin (FHA) and pertussis toxin (PTx):

- 1- FHA** is a large protein that forms filamentous structures on the cell surface. Antibodies against FHA provide protection against infection.
- 2- The PTx** is both secreted into the extracellular fluid and cell bound. The cell-bound toxin functions as an adhesin. Thus, pertussis toxin is clearly an important virulence factor both in the colonization and the toxæmic stages.

B- Toxæmic stage begins gradually with prolonged and paroxysmal coughing that often ends in a characteristic inspiratory gasp (whoop). During this stage, *B. pertussis* can rarely be isolated, and antimicrobial agents have no effect on the progress of the disease. This stage is mediated by a variety of toxins produced by *B. pertussis*:

1- Pertussis toxin (PTx): PTx has two components A and B. It is similar to the A-B structure-function model of cholera toxin. PTx has a potent adenylate cyclase activity that reduces phagocytic activity locally and helps the organism to initiate infection. Systemic effects of the toxin include lymphocytosis and increased sensitivity to histamine (resulting in increased capillary permeability, hypotension and shock). This toxin can be inactivated and converted to toxoid for use in vaccines.

2- Tracheal cytotoxin (TCT): TCT is not a classic bacterial exotoxin, since it is not composed of protein, but is a peptidoglycan fragment. The toxin kills ciliated respiratory epithelial cells. It also stimulates release of IL-1, and so causes fever.

not protein But it is peptidoglycan
Kills ciliated respiratory epithelial cells
↑ fever

Whooping Cough (Pertussis)

transmitted by droplet

3 stages
 ① Catarrhal
 ② Paroxysmal
 ③ Convalescent

It is an acute respiratory disease transmitted by droplet. The incubation period is about 7-10 days. Classical pertussis has 3 stages, the catarrhal, paroxysmal and convalescent stages. The most prominent and serious symptom is the severe paroxysmal cough. Complications occur such as cyanosis, secondary infections as pneumonia and otitis media. Encephalopathy is the most serious complication. The typical disease usually occurs in unimmunized infants. Adult pertussis also occurs because vaccine-induced immunity and naturally acquired immunity are not long-lived.

not productive
 Cough
 = dry
 = no sputum

Laboratory diagnosis

A. Specimens: Nasopharyngeal (NP) secretions obtained by pernasal swabs, aspiration or cough plate.

B. Direct detection in NP secretions by:

- 1- The direct fluorescent-antibody (DFA) test.
- 2- Nucleic acid detection by PCR.
- 3- Direct Gram stain is useless.

C. Cultivation: The following characters differentiate *B. pertussis* from other species of the genus:

- 1- The organism does not grow on blood agar or MacConkey agar but requires the special media Bordet-Gengou agar and charcoal blood agar.
- 2- Growth is slow and requires incubation for 5-7 days at 37°C in ambient air.
- 3- Colonies have characteristic morphology (mercury-droplet appearance).

no Blood no chocolate no MacConkey
 only → Bordet-gengou & charcoal blood agar

slow grower

D. Identification: Colonies that are oxidase-positive, urease-negative, and does not ferment carbohydrates can be readily identified by the *B. pertussis* antiserum using DFA test or agglutination, or by PCR.

E. Serology: it is of little help because antibodies are not detectable until the third week of illness.

3rd week

Antimicrobial susceptibility

Antibiotic treatment is highly effective if given early and helps eradication of the organism and prevents infectivity. Erythromycin is the drug of choice. The other choice is trimethoprim-sulfamethoxazole.



Whooping cough vaccines

A- Killed whole cell vaccine: It is a part of the DPT vaccine (The P in DPT stands for pertussis cells). Unfortunately, about 20% of the children that receive the whole cell vaccine experience mild side effects. In a very small number of cases severe or irreversible brain damage may occur. Thus, the value of the whole cell vaccine has been questioned.

B- Acellular vaccines: New acellular vaccines have been developed from combinations of pertussis toxoid (genetically inactivated toxin), filamentous haemagglutinin and other virulence factors. The acellular pertussis vaccine has fewer side effects than the whole cell vaccine and is currently recommended for use combined with diphtheria and tetanus toxoids as DTaP.

Why Acellular is more preferred?

Why Not recommended whole killed?

Because Acellular less side effects

Facultative intracellular
Pathogen
→ Brucellosis

Chapter 17

BRUCELLA

"I see the
egg cell
Zoonotic"

Brucella species are normal flora of the genital and urinary tracts of many animals. Brucellosis is a zoonotic disease. The organisms are facultative intracellular pathogens and cause brucellosis in man and animals. Species or biovars are named after their host or the disease they produce:

1. *B. abortus* infects cattle; the placenta is infected, causing abortion in the animal.
2. *B. melitensis* infects sheep and goats.
3. *B. suis* infects pigs.

B. abortus
B. melitensis
B. suis → الـسبحان
@ Shigella
dr, y!

Morphology

Members of the genus *Brucella* are very small Gram-negative bacilli (coccobacilli), non-motile, non-capsulated and non-sporeforming.

Culture

Brucella is aerobic and grows on media enriched with serum or blood e.g. serum dextrose agar. *B. abortus* requires 10% CO₂ for primary growth. The cultures of the different *Brucella* species show variable sensitivity to basic fuchsin and thionine dyes. This property can be used to differentiate such species.

Biochemical reactions

Brucella organisms are usually catalase-positive, oxidase-positive, rapid urease producers and reduce nitrates. *B. abortus* and *B. suis* produce H₂S.

Catalase +ve
oxidase +ve
urease +ve
reduce nitrates

Antigenic structure

The *Brucella* species have two distinguishing antigens associated with the LPS of *Brucella* cell wall. They are designated A and M and can be detected by agglutination tests using monoclonal antibodies.

Brucellosis (Undulant Fever or Malta Fever)

Mode of transmission

Domestic animals serve as the reservoir. Transmission occurs by:

1. Ingestion of unpasteurized milk, milk products and meat from infected animals.
2. Direct contact through skin abrasions and mucous membranes during handling of infected animals as an occupational disease (e.g. in butchers, farmers and veterinarians).
3. Inhalation during handling of *Brucella* cultures in the laboratories.

← ingestion
direct contact with infected subject through skin abrasions
inhalation during handling culture

Milk diseases

T.B.
Brucellosis



Clinical manifestations

Key Case
Bodyache
drink milk

Brucellosis may be presented acutely or subacutely or as local disease. Systemic symptoms include fever, which is usually prolonged and intermittent (undulant), chills, weakness, malaise, body aches, sweating and headache. Brucellosis may also involve the liver, heart (endocarditis) and central nervous system (meningitis). In its subacute form, it may resemble tuberculosis.

Immunity

Brucella is able to survive intracellularly in phagocytic cells. Therefore, the host defence depends on cellular immunity. Delayed hypersensitivity reactions are responsible for the granuloma and abscess formation in bones or any organ. Antibody response to brucella includes production of IgM, IgG and IgA.

Laboratory diagnosis

A. Specimens: Blood or bone marrow during the acute illness, joint fluid of patients with arthritis, pus from abscesses or CSF from CNS affections.

B. Direct detection in clinical materials:

1- PCR.

2- Gram stain is useless.

C. Cultivation

1- Blood culture: a blood sample (5-10 ml) is added to 50-100 ml broth (heart infusion or brucella broth). Subcultures are done on serum dextrose agar or blood agar every 48 hours and growing organisms are identified as mentioned below. Since the organism is difficult to isolate on initial cultivation, blood cultures should not be regarded negative except after prolonged incubation for 6-7 weeks in presence of 10% CO₂.

2- Direct inoculation of other specimens onto serum dextrose agar, blood agar or chocolate agar and incubation in 10% CO₂.

D. Identification

Growth is examined by Gram stain. Identification of *Brucella* is carried out on 2 levels, genus and species:

- 1- The growth is identified as a *Brucella* genus if:
 - a. It is catalase-positive, oxidase-positive and rapid urease producer.
 - b. Reduces nitrates.
 - c. Agglutinates with *Brucella* monoclonal antibodies to A and M antigens.
- 2- Species can then be identified according to CO₂ requirements, H₂S production and dye sensitivities (Table 9).

Table (9): Differentiation of *Brucella* species

Organism	Inhibition of growth by		CO ₂ requirement for growth	H ₂ S production	Dominant antigen
	Basic fuchsin	Thionine			
<i>Br. abortus</i>	No	Yes	Yes	Yes	A > M
<i>Br. melitensis</i>	No	No	No	No	M > A
<i>Br. suis</i>	Yes	No	No	Yes	A = M

FLP
2011

E. Serologic tests: Two serum samples should be collected for serological diagnosis: One at the onset of illness and the second after 14-21 days.

1- Standard tube agglutination test (STAT) for total antibody (IgG + IgM), is the commonest test used for diagnosis of brucellosis. The STAT detects antibodies to the three *Brucella* spp. A single titre of ≥ 160 or a fourfold rise in titre or greater is considered significant. False negative results may be obtained. Therefore, the following procedures should be implemented during performance of the test:

a- Wide range of serum dilutions (start with 1:20 up to 1:5120) in order to eliminate the **prozone effect** that is commonly encountered in cases of brucellosis.

prevent prozone

b- Anti-Brucella Coomb's test (or mixed agglutination reaction; MAR) should be done for negative results to eliminate the effect of **incomplete or blocking antibodies (IgA)**, which are produced in certain cases and obscure agglutination in real cases of brucellosis.

prevent incomplete blocking antibody

2- ELISA for IgG or IgM.

F. Brucellin test

It is a skin test similar to tuberculin test and is based on delayed type hypersensitivity. It is of diagnostic help in certain situations.

Prevention

1. Pasteurization of milk and its products.
2. Control of infections in animals.
3. Live attenuated vaccine for cattle.

Treatment

Treatment of brucellosis requires combination of antibiotic therapy for a prolonged period due to the intracellular residence of the organisms. Tetracyclines, aminoglycosides, rifampin and trimethoprim-sulfamethoxazole have been all used with success.

Combined to prevent drug resistance

No Vaccination

Chapter 18

LEGIONELLA

The genus *Legionella* contains about 40 species, all normally live in fresh water, and all are pathogenic for humans. *L. pneumophila* is the major pathogenic species. It causes Legionnaire's disease and Pontiac fever.

Morphology: *Legionella* species are motile, faintly stained Gram-negative bacilli.

Culture: *Legionella* species are aerobic fastidious bacteria. They do not grow on ordinary blood agar, but on a medium supplemented with growth factors named buffered charcoal yeast extract (BCYE) agar.

Legionnaire's Disease and Pontiac Fever

- Legionnaire's disease or legionellosis is an atypical bacterial pneumonia.
- Pontiac fever is a mild, flulike form of *Legionella* infection that does not result in pneumonia.

Reservoir

1. Water and soil.
2. within free-living amoebae living in environmental water.
3. in biofilms.

Mode of transmission

- Inhalation of contaminated water aerosols.

There is no person-to-person transmission.

Infections can occur both as community-acquired or nosocomial. Outbreaks of pneumonia in hospitals have been attributed to the presence of the organism in water taps, sinks, showers and air-conditioners.

Pathogenesis

Aside from endotoxin, no other virulence factors are known. Predisposing factors include smoking, high alcohol intake, old age and immunosuppression.

The organism replicates intracellularly, therefore cell-mediated immunity is an important host defence.

Diagnosis

- Microscopy: using silver stain or fluorescent antibody.
- Culture: on BCYE agar.
- Detection of urinary antigen: provides rapid diagnosis.
- Detection of rising antibody titre in patient's serum.

Treatment

Azithromycin or erythromycin. Rifampin can be added in severe cases.

Legionnaire disease
Pontiac fever
P. pneumophila

Transmit by Inhalation

Virulence = endotoxin

No person to person

Why not all person Inhale Contaminated aerosol get sick?

Bec it is weak virulence only endotoxin require pred.



SPIROCHAETES

G.R. Motile even they have no flagella because have axial filament endoflagella

Spirochaetes are thin-walled, flexible, spiral rods. They are motile through the undulation of axial filaments (endoflagella) that lie under the outer sheath. Three genera of spirochaetes cause human infection: (1) *Treponema*, which causes syphilis and the nonvenereal treponematoses; (2) *Borrelia*, which causes relapsing fever and Lyme disease, and (3) *Leptospira*, which causes leptospirosis. Treponemes and leptospirae are so thin that they are seen only by dark-field microscopy, silver impregnation or immunofluorescence. Borreliae are larger, accept Giemsa and other blood stains, and can be seen by the light microscope.

Treponema pallidum

anaerobic

Morphology

T. pallidum is a slender spirochaete (0.2 μm in diameter). It has regular coils with pointed ends. The organism shows a characteristic rapid corcscrew (forward and backward) movement. Unstained organisms are not visible by the standard light microscope because of the small cell diameter. *T. pallidum* is usually stained by silver stains (e.g. Fontana stain).

Sample = chancres exudate

Isolation procedures (Cultivability)

T. pallidum cannot be cultivated in vitro. It can be propagated by inoculation of positive specimens into rabbit testicles.

G.R. no cultivation

Habitat

T. pallidum is a human parasite. It has no animal or environmental reservoirs.

Susceptibility to physical and chemical agents

Any contact with air, antiseptics or sunlight will kill the microbe. Its fastidious nature may account for its obligate parasitism and rapid death outside the host.

because very fragile

Mode of transmission

1. Sexual exposure leads to venereal syphilis. The bacteria usually enter the body during sexual intercourse.
2. Transplacental leading to congenital syphilis.
3. Fresh blood transfusion: *T. pallidum* is not transmitted by stored blood because it dies when stored at 4°C within 3-5 days.

because v. fragile

Syphilis

Pathogenesis and clinical manifestations

Many people infected with *T. pallidum* do not have any symptoms for years, but remain at risk for late complications of syphilis if they are not treated. Classically, untreated syphilis has three distinct stages and a latent stage between the second and third stages.

Primary syphilis This is usually manifested as a single **hard, painless ulcer** called **chancre**. The incubation period is 10-60 days (average 21 days). The chancre represents the primary site of initial multiplication. It usually appears on the penis, labia, cervix, anorectal region, or around the mouth. The regional lymph nodes also become enlarged. The chancre heals within 4-6 weeks, even without treatment.

Secondary syphilis This stage has four cardinal features:

1. Generalized skin rash.
2. Mucous patches in mouth and throat.
3. Chondyloma lata (moist papules) form around the genitals or anus.
4. Generalized lymphadenopathy.

The symptoms usually last 3-6 months and disappear spontaneously.

The Latent (hidden) stage: There are neither symptoms nor lesions during this stage, and the serology is positive. This stage can range from a few months to a lifetime.

Tertiary syphilis This may occur in about 40% of untreated cases. This stage is characterized by gumma formation in the internal organs and bones and syphilitic lesions that may lead to cardiovascular syphilis and neurosyphilis (tabes dorsalis). Serology is positive.

Congenital Syphilis: In utero infections can lead to a stillbirth (a baby born dead) or giving birth to a baby who dies shortly after birth, or latent infections. Most infected infants are born without symptoms, but within a few weeks they develop rhinitis followed by a widespread rash. There is no primary stage in congenital syphilis. Late bony destruction and cardiovascular syphilis are common in untreated infants.

Direct detection enough for diagnosis

- ① Acute gonorrhea
- ② Leprosy
- ③ epidemic cases of cholera
- ④ 1918-19 influenza pandemic

⑥ relapsing fever
⑦ venereal angina

Laboratory diagnosis of venereal syphilis

Each stage of syphilis has a particular testing requirement as follows:

1. **Primary stage:** Serous fluids from chancre contain numerous treponemes. Therefore, dark-field microscopy or direct immunofluorescence of serous fluids from chancre is the method of choice for diagnosis. Antibodies usually do not appear until 1 to 4 weeks after the chancre has formed. Therefore, serologic tests for syphilis are not reactive except late.
2. **Secondary stage:** All serologic tests for syphilis are reactive. Also treponemes can be directly detected in the mucous patches and chondyloma lata.
3. **Tertiary syphilis** is diagnosed only by serologic tests.

Antibodies not appear until 1-4 week after chancre
So serologic tests not reactive except late

Thus two categories of methods can be used for diagnosis of syphilis:

- ⇒ 1. **Direct detection** is used for chancre, mucous patches and chondyloma lata.

It includes:

- Dark-field microscopy of wet unstained preparation which detects the characteristic motility. *forward + backward movement corkscrew*
- Direct immunofluorescence: Smears prepared from syphilitic lesions are stained with fluorescein-labelled specific treponemal antibodies. This method is highly sensitive and specific.
- PCR.

- ⇒ 2. **Serologic tests of syphilis:** Serum is the specimen of choice CSF is used in suspected neurosyphilis. They include:

- a- **Non-treponemal tests** which are non-specific tests used for screening.
- b- **Treponemal tests** which are specific tests used for confirmation.

CSF CSF
Reagin
= heterophil antibody

Non-treponemal tests

- The antigen is composed of an alcoholic solution containing measured amounts of cardiolipin, cholesterol and lecithin to produce standard reactivity.
- They include the following flocculation tests:
 - 1- The venereal disease research laboratory (VDRL). This test is highly valuable. The flocculation is seen by microscopic examination.
 - 2- The rapid plasma reagin (RPR) card test. The flocculation is seen with the naked eye. This test can be applied on plasma as well as serum and CSF.
- These tests measure antibodies to lipoidal material released from damaged host cells as well as from treponemes. These cross-reacting (heterophil) antibodies (known as reagin) are also produced in other conditions like autoimmune diseases, pregnancy, leprosy, immunization and viral infections. Thus, false reactivity is expected during diagnosis of syphilis. Therefore, reactivity for syphilis must be confirmed by one of the specific treponemal tests.
- These tests are inexpensive, rapid and simple to perform. Therefore they are used for screening. They are also used for follow up of treatment, since reactivity declines or disappears within 6-18 months of effective therapy.

Antigen is corkscrew

if false -ve
not true +ve always
done before screening first not expensive follow up bec.
reagin declines & disappears

11

y VDRL +ve & TPHA -ve
= -ve for syphilis
& +ve may be due to cross reaction with other diseases with (reagin of syphilis)

Treponemal tests

- The antigen is *T. pallidum*.
- They include:
 - 1- The fluorescent treponemal antibody absorption (FTA-ABS) test.
 - 2- *T. pallidum* haemagglutination assay (TPHA).
 - 3- *T. pallidum* immobilization (TPI) test.
 - 4- The EIA and the Western blot.
- These tests detect antibodies specific for cellular components of the organism.
- They are not screening tests because they are expensive and difficult. However, because they are specific, they are used in confirming or ruling out reactive non-treponemal test result.
- They remain reactive for life.

Serologic testing for syphilis must start with the non-treponemal tests. Reactive results only are then confirmed by the specific treponemal tests.

Diagnosis of congenital syphilis

It is done by detection of treponemal IgM antibodies by EIA or Western blot.

→ not IgG

Treatment, prevention and control

Penicillin is the drug of choice for treatment of syphilis. Tetracycline, erythromycin and chloramphenicol can be used as alternative antibiotics for patients allergic to penicillin. Only penicillin or chloramphenicol can be used for patients with neurosyphilis. Syphilitic pregnant mothers should be adequately treated to prevent congenital syphilis.

* relapsing fever
* Lyme disease

Borrelia

microaerophilic

Borrelia can be pathogenic for humans, domestic animals, rodents and birds. Human borreliosis includes **relapsing fever** and **Lyme disease**. All borreliae are transmitted by blood-feeding arthropods. Ticks transmit all known species of *Borrelia* except *B. recurrentis* which is transmitted by the human body louse.

Morphology

Borreliae are highly motile organisms with flat and helical waves. Borreliae are best visualized with Giemsa's stain. Some are Gram negative.

Cultural characteristics

Borreliae are microaerophilic slowly growing spirochaetes. They grow on a highly enriched medium named BSK II medium, at 30 to 35°C.

Relapsing Fever

Caused By *Borrelia*

3-7 relapses

G.R. of recurrence?

bec. of
genetic
rearrangement
+
mutation
+
variation

Relapsing fever is a febrile, septicaemic disease with sudden onset after an incubation period of 2 to 15 days. Fever persists for 3 to 7 days and is followed by an afebrile interval of several days to several weeks. Thereafter, as many as 3 to 7 relapses may occur. Relapses occur as a result of antigenic variations in the causative *Borrelia* spp. Such variations are due to programmed rearrangement of bacterial DNA encoding surface proteins. The new antigenic variants will not be destroyed by antibodies directed against the original infecting one. Thus, the patient clinically improves until the new clone multiplies sufficiently to cause another relapse.

Transmission and pathogenesis

all *Borrelia* Transmit by Ticks
except *recurrentis*
By Louse

There are two forms of relapsing fever:

1. **Louse-borne (epidemic)** relapsing fever caused by *B. recurrentis*. It has worldwide distribution. Man is the only reservoir. It is transmitted from man to man by crushed human body louse.
2. **Tick-borne (endemic)** relapsing fever caused by *B. duttoni* and *B. hermsii*. Rodents and small animals are the main reservoir. It is transmitted by ticks.

Laboratory diagnosis

A- Specimens: Blood.

B- Direct detection

- 1- During acute phases:
 - a) Wet preparations from the patient blood are examined by dark-field or phase contrast microscopy.
 - b) Blood films stained with Giemsa or Leishman are examined by conventional light microscopy.
- 2- In mildly infected patients: Blood sample should be centrifuged first to concentrate the organisms before examination of wet and stained smears.

Direct is enough in acute phase

C- Cultivation and enrichment in the mouse: Injection of the patient's blood into a white mouse followed by inoculation of the infected mouse blood onto BSK II medium is required. The culture is monitored for spirochaetes by dark field microscopy for 4 to 6 weeks.

D- Identification by DNA hybridization.

E- Serologic tests: They lack specificity due to antigenic sharing among borreliae and with other spirochaetes.

G.R. not specific bec.



Lyme Disease

By Ticks Transmission
by *Borrelia burgdorferi*

The disease is caused by *B. burgdorferi* which is transmitted by ticks bite. Lyme disease is an inflammatory disorder that usually begins with a characteristic skin lesion called erythema migrans, accompanied by headache, stiff neck, myalgias, arthralgias or swelling of the lymph nodes. Later, some patients develop meningoencephalitis, myocarditis or chronic arthritis.

Leptospira

By *Leptospira interrogans*
= (*icterohaemorrhagiae*)
Jaundice Haemorrhage

The genus *Leptospira* consists of aerobic spiral-shaped bacteria with hooked ends. These organisms are the causative agents of leptospirosis, a zoonosis with worldwide distribution. Pathogenic serovars belong to the species *Leptospira interrogans*. *L. interrogans* serovar *icterohaemorrhagiae* is the most virulent and is responsible for the severest form of leptospirosis in humans.

Morphology

The organisms are very thin motile helical rods of 0.1 μ m in diameter. Usually, one or both ends of the cells are hooked. They stain faintly negative upon Gram staining, but they are best viewed by dark-field or phase contrast microscopy.

Leptospirosis Weil's Disease (Infectious Jaundice)

Leptospirae have a worldwide distribution. They may be free-living or may live in association with human or animal hosts. The organisms are excreted in the urine of infected animals, e.g. dogs, rats, swine and cattle and may survive for days to months in fresh water, soil or mud.

Mode of transmission

Rodents, dogs, swine and cattle act as reservoirs for the leptospirae. Transmission occurs primarily via infected urine, which contaminates stagnant water sources. Outbreaks occur in swimmers while occupational exposure may result in infection of rice farmers, sugarcane workers and military personnel.

Clinical manifestations

Leptospirosis exhibits a great variety of clinical manifestations, ranging from a mild self-limiting febrile illness (most patients) to a fulminating fatal illness associated with hepatorenal failure (Weil's disease). The incubation period is usually 7 to 14 days. Leptospirosis is typically a biphasic illness with quiescent period in between:

- †
- ① **The initial septicaemic phase** is characterized by icteric leptospirosis (jaundice). It lasts for 4 to 7 days.
 - ② **The second phase:** There is meningitis (up to 40% of patients) and leptospiruria (>95% of patients).

Laboratory Diagnosis

- Schmugella fever*
- A- **Specimens:** Blood is used for culture during the first week. After the first week, leptospirae could be isolated from the urine and CSF.
 - B- **Direct detection:** A presumptive diagnosis of leptospirosis can be made by 1) direct microscopic methods by either dark-field microscopy or direct fluorescent antibody assay or silver stains. 2) PCR for the rapid detection of leptospira DNA in specimens.
 - C- **Cultivation:** Suitable media include the protein-rich semisolid media, such as Fletcher's or Stuart's medium. Cultures should be held aerobically at 30°C. Growth usually appears within 1 to 2 weeks.
 - D- **Identification** by the microscopic agglutination test or PCR.
 - E- **Serologic tests:** ELISA for total antibody and IgM proved to be highly sensitive.

Antibiotic susceptibilities

Penicillin and doxycycline are the drugs of choice.

Fusospirochaetal Disease

direct smear
endogenous transmission

Under certain circumstances, particularly injury to mucous membranes, nutritional deficiency or concomitant infection (e.g. with herpes simplex virus), the normal spirochaetes of the mouth, together with anaerobic fusiform bacilli (fusobacteria) find suitable conditions for multiplication. They increase in numbers causing:

- ① **Trench mouth:** It is a condition of acute necrotizing ulcerative gingivitis (ANUG).
- ② **Vincent's angina:** Fusospirochaetal infection of the pharynx with pseudomembrane formation, similar to diphtheria and follicular tonsillitis.

Laboratory diagnosis

Gram stained smear from the pseudomembrane shows large number of Gram-negative fusiform bacilli and spirochaetes in association with pus cells and other organisms.

Chapter 20

MYCOPLASMA AND UREAPLASMA

*No cell wall © $\rightarrow$ small size
 $\rightarrow$ resist penicillin
 $\rightarrow$ pleomorphic (variable shape)

Characteristic Features

1. These two genera are **bacteria without cell wall**. The lack of a cell wall renders these organisms:
 - a- Resistant to the beta-lactam antibiotics (e.g. penicillins and cephalosporins).
 - b- Unstainable by the Gram stain but readily stained by Giemsa stain.
 - c- Variable in shape (pleomorphic).
2. Their contents are enveloped by a cell membrane.
3. They are the only bacteria that contain sterol in the cell membrane.
4. They are the smallest bacteria (0.2-0.3 μm) that can be grown on cell-free media. They require serum enriched medium containing sterols. They are facultative anaerobes and fastidious in nutrition.

Table (10): *Mycoplasma* species, diseases and modes of transmission in humans

Species	Disease	Transmission
<u><i>Mycoplasma pneumoniae</i></u>	1. <u>Atypical pneumonia</u> 2. Bronchopneumonia 3. Acute pharyngitis 4. Anemia (cross-reaction of anti-mycoplasma IgM with patient erythrocytes) 5. Septic arthritis	Inhalation of <u>respiratory droplets</u> among close-contact groups
Genital mycoplasmas 1. <i>M. hominis</i> 2. <i>M. genitalium</i> 3. <i>Ureaplasma urealyticum</i>	1. <u>Nongonococcal urethritis in men</u> 2. <u>Neonatal sepsis in prematures</u> 3. Acute pyelonephritis, pelvic inflammatory disease (PID) and postabortal or postpartum fever 4. Septic arthritis	1. <u>Sexual</u> 2. <u>Vertically</u> from mother to foetus

Laboratory diagnosis

A- Specimens include respiratory secretions and genital discharge.

B- Direct detection

- 1- Gram staining is not useful.
- 2- Antigen detection by immunofluorescence using specific antibodies.
- 3- PCR.

فريجيدو: (P) fried egg appearance

C- Cultivation and identification

- *Mycoplasma pneumoniae*: After incubation for about 3 weeks, colonies on the serum enriched medium appear very small with a characteristic "fried egg" appearance. Identification is done by Giemsa staining, immunofluorescence or PCR.
- *Ureaplasma urealyticum* can be detected in culture within 24-48h. It is identified by its characteristic colony morphology and urease production.

D- Serology

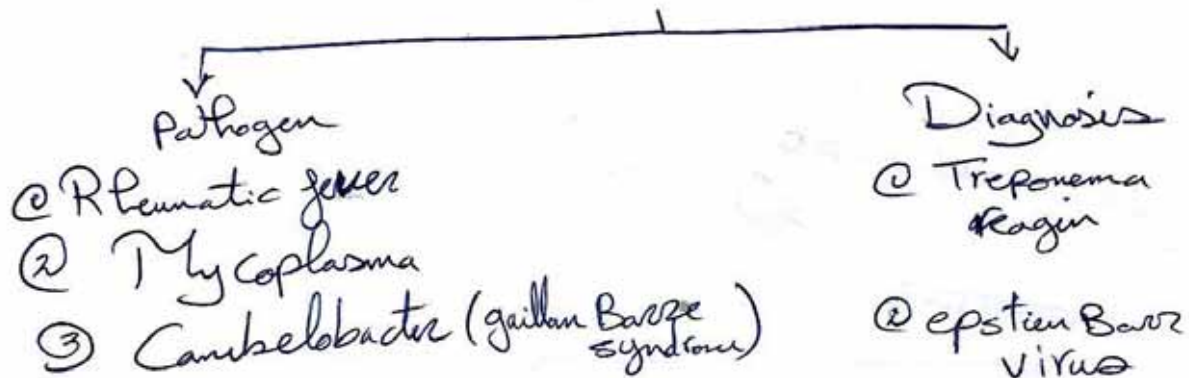
- 1- Specific tests is the most accurate:
 - Detection of IgM antibodies by EIA.
 - Detection of IgG by EIA; a fourfold rise in antibody titre is diagnostic.
- 2- Nonspecific test: A titre of $\geq 1:128$ of cold agglutinins is indicative of recent infection. Cold-agglutinins are IgM antibodies that agglutinate group O red cells at 4°C but not at 37°C . Cold agglutinins are found to be positive in 50% of cases of *M. pneumoniae* infections, but positive results occur also in some viral infections, malaria and haemolytic anaemia.

Treatment

New quinolones such as trovafloxacin and sparflaxacin are the drugs of choice for all mycoplasmas.

Dg = Ab. produced against antigen and cross react with another similar Antigen

Heterophil phenomenon



Chapter 21

CHLAMYDIA

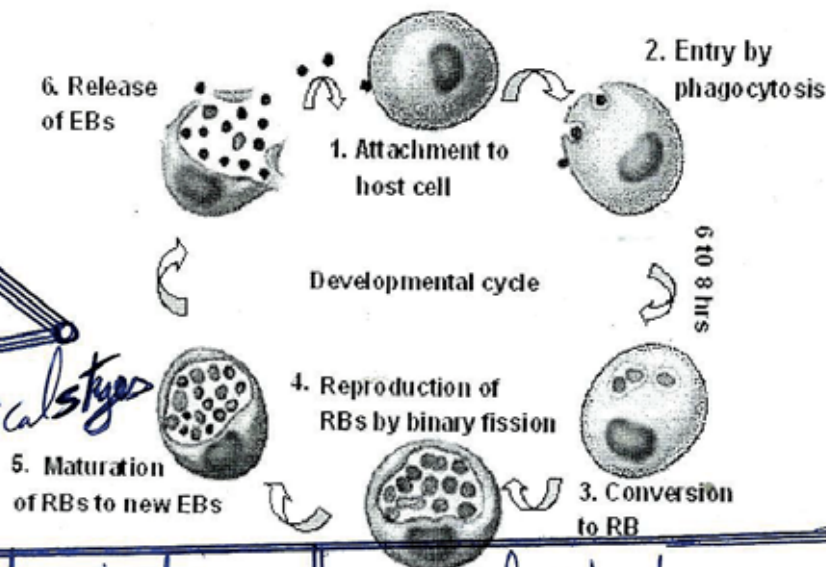
G.R. it's not virus
Ans. p. 102

Characteristic Features

1. *Chlamydia* is a genus of **very small obligate intracellular bacteria**. They lack the ability to produce sufficient energy to grow independently and therefore can grow only inside host cells.
2. They have a lipopolysaccharide cell wall similar to Gram-negative bacteria.
3. They are stained poorly with Gram but readily by **Giemsa stain**.
4. They can be grown in the **yolk sac of chick embryo** or **tissue culture**. The presence of the organism is detected by staining for intracytoplasmic basophilic inclusion bodies.
5. They multiply by **binary fission** with a **biphasic developmental cycle**.

Developmental cycle

This cycle explains pathogenesis of diseases caused by *Chlamydia*. There are two morphological forms, **elementary bodies (EBs)** and **reticulate bodies (RBs)**. EBs are small (0.3 μm), spherical, **infectious** particles, metabolically inert, can survive extracellularly but do not replicate. The EB attaches to the host cell and enters by phagocytosis. Within the cell it increases in size and becomes reticulate body (0.8-1.2 μm) which is metabolically active. The RB divides by binary fission, yielding pleomorphic organisms which mature to new EBs. Release of EBs follows rupture of the cell to infect new cells. Within the infected cells, the site of replication appears as an inclusion body which can be stained and visualized microscopically. These inclusions are useful in diagnosis.



Chlamydia
2 Morphological stages

elementary body	reticulate body
spherical	reticular
0.3	0.8 - 1.2
extra cell	intra cell
metab inert	metab active +



الصفحة
تذاكر من الجداول

Chlamydiae that infect humans are divided into three species (Table 11):

1. *Chlamydia trachomatis*: has 15 serotypes.
2. *Chlamydophila pneumoniae*: has 1 serotype.
3. *Chlamydophila psittaci*: has 1 serotype.

Diseases caused by *Chlamydia trachomatis*

I. Non-sexually transmitted diseases:

A. Ocular infections:

- 1- **Trachoma** is a major worldwide disease, especially among young children. It manifests as chronic keratoconjunctivitis with scarring which may lead to blindness. North Africa and the Middle East, including Egypt, are particularly affected. The serotypes involved are A, B and C. Humans are the only reservoir and transmission commonly occurs by fingers, fomites and flies mostly during hot and dry climates.
- 2- **Inclusion conjunctivitis**: It is the most common cause of neonatal conjunctivitis. It is caused by serotypes D through K. Man is the only reservoir and person-to-person spread is the mode of infection in either neonates (during birth) or adults (swimming pool).

B. Neonatal pneumonia in neonates born vaginally to infected mothers.

II. Genital tract infections, they are sexually transmitted diseases:

- 1- **Nongonococcal (NGC) urethritis**, cervicitis, salpingitis, proctitis and epididymitis: They are typical *Chlamydia* diseases caused by serotypes D through K. Chronic or repeated infections can cause pelvic inflammatory disease (PID), infertility, sterility and ectopic pregnancy.
- 2- **Lymphogranuloma venereum (LGV)** is caused by L1, L2 or L3 serotypes, where an initial papular lesion gives rise to suppurative lymphadenopathy.

Diseases caused by *Chlamydophila* species

- *Chlamydophila pneumoniae* causes an atypical pneumonia and has recently been associated with atherosclerosis and heart disease, adult asthma and Alzheimer's disease.
- *Chlamydophila psittaci* causes psittacosis which is an atypical pneumonia. It is transmitted via inhalation of particles contaminated from bird dried faeces or respiratory secretions.

Table (11): Chlamydiae that infect humans

Species	Serotype	Disease	Transmission
<i>Chlamydia trachomatis</i>	A, B, C	<u>Trachoma</u> → <u>blindness</u> Endemic in Egypt	<u>fingers, flies, fomites</u>
	From D to K	<u>Inclusion conjunctivitis</u> in adults and neonates	Swimming pool During birth
		<u>Neonatal pneumonia</u>	During birth
		<u>NGC urethritis</u> <i>non gonococcal</i>	<u>Sexual</u>
	L-1, L-2, L-3	<u>LGV</u> <i>lymphogranuloma venereum</i>	
<i>Chlamydophila pneumoniae</i>	TWAR	<u>Atypical pneumonia</u> <u>Atherosclerosis</u> , heart disease, adult asthma and Alzheimer's dis	<u>Inhalation of respiratory droplets from humans</u>
<i>Chlamydophila psittaci</i>	Unidentified	<u>Psittacosis</u> = <u>atypical pneumonia</u> .	<u>Inhalation of particles contaminated from bird dried faeces or respiratory secretions</u>

NGC urethritis: Nongonococcal urethritis, LGV: Lymphogranuloma venereum

Laboratory diagnosis

A. **Specimens** are taken from conjunctiva, urethra, cervix, vagina, pus, sputum... etc.

B. Direct detection

- 1- Microscopic examination of Giemsa stained smears for detection of the basophilic inclusion bodies. This method is insensitive, non-specific and requires good experience.
- 2- Antigen detection by immunofluorescence or by ELISA. This method is more specific, reliable and rapid.
- 3- Nucleic acid detection by DNA probes or PCR.

C. **Cultivation and identification:** Specimens are inoculated in the yolk sac of chick embryo or tissue culture. The presence of the organism is detected by staining for intracytoplasmic basophilic inclusion bodies, immunofluorescence or by ELISA.

D. **Serological tests:** Detection of specific IgM or significant increase of IgG antibodies in the patient serum by CF test or by ELISA.

E. **Frei skin test:** similar to tuberculin test. It is used for diagnosis of LGV infection.

Treatment and prevention

The antibiotic of choice is azithromycin or tetracycline in adults. Azithromycin (or erythromycin) is recommended for babies because tetracycline causes discolouration of their teeth. Prevention involves improvement in personal hygiene and simultaneous treatment of all sexual partners.



Chapter 22

Rickettsia
+
Chlamidia

COXIELLA

2 Antigenic phases
II acute
I chronic

C. burnetii is the only species of *Coxiella* genus. It causes Q (Query) fever, an extremely common infection, which may occur in an acute or chronic form.

Characteristic features

Same

1. Short obligate intracellular bacteria, with a Gram-negative cell wall structure.
2. Does not stain with Gram stain, but readily with the Gimenez method.
3. Can be grown in cell culture, embryonated eggs, mice and guinea pigs.
4. Displays an intracellular developmental cycle, leading to the formation of spore-like forms which are more resistant to dryness and only killed after prolonged exposure to high concentrations of formalin.
5. Displays 2 antigenic phases. **Phase I** is the natural phase found in infected animals, arthropods or humans and it is highly infectious. In contrast, **phase II** is not very infectious.

Transmit By ingestion of Milk @ air born aerosols

Q Fever

2011

By *Coxiella burnetii*

Q fever is a zoonosis. The reservoir includes many mammals, birds and arthropods such as ticks. However, cattle, sheep and camels represent the most frequent source of human infection. Q fever is usually an occupational hazard. People at high risk include farmers, abattoir workers and veterinarians as well as laboratory personnel.

Pathogenesis and clinical presentations

C. burnetii infections are mainly transmitted by:

1. **Airborne:** *C. burnetii* is acquired via inhalation of animal **aerosols** (especially from sheep faeces, placenta or urine exposure).
2. **Ingestion** of milk from infected animals.

Infection usually occurs via the **respiratory** route and the alveolar macrophages are the primary cells infected during acute Q fever. Kupffer cells in the liver are also susceptible and may be infected via the **bloodstream** or from the **digestive** route. The incubation period of Q fever may range from 1 to 3 weeks. It may present as a self-limited flu-like illness, acute Q fever or chronic Q fever (Table 12). However, in the majority of cases, infection remains asymptomatic.

Table (12): Comparison between acute Q fever and chronic Q fever

	Acute Q fever	Chronic Q fever
Duration	Less than 6 months	Longer than 6 months
Main manifestations	1. Atypical pneumonia 2. Hepatitis <i>Jaundice</i>	1. Endocarditis 2. Prolonged fever
Occasional manifestations	1. Meningoencephalitis 2. Myocarditis, pericarditis	1. Osteomyelitis 2. Hepatitis
Predisposing factors	None	Immunocompromised patients or pregnant
Conventional blood culture	Negative <i>-ve</i>	Negative <i>-ve</i>
Diagnostic serology	Phase II <i>C. burnetii</i> antibody	Phase I <i>C. burnetii</i> antibody
Treatment	Tetracycline for several days	Tetracycline for several months

Laboratory diagnosis

A. Specimens: Blood, CSF and a variety of tissues (e.g. liver biopsy).

B. Direct detection

- 1- Immunofluorescence to detect *C. burnetii* in tissues.
- 2- PCR.

C. Cultivation and identification

- 1- Intraperitoneal inoculation of guinea pigs or mice.
- 2- Tissue culture.

C. burnetii can be seen in the animal tissues or culture by microscopy and confirmed by indirect immunofluorescence (IFA) or PCR.

D. Serology

- Q fever diagnosis depends upon serology because culture and molecular techniques have low sensitivity and are available only in reference laboratories.
- Two serum samples are collected: an early sample during the acute-phase and a convalescent sample after 2-4 weeks.
- A fourfold rise or greater in antibody titres is considered diagnostic.
- Serology allows the differentiation of acute and chronic Q fevers. The IFA test is the test of choice:
 - a. An IgG anti-phase II antibody titre of ≥ 200 is significant of acute Q fever.
 - b. Presence of anti-phase I antibody is significant of chronic Q fever.

Vaccine prophylaxis

Q fever vaccine used in humans consists of killed, purified phase I *C. burnetii* whole cells. It is usually given to people at risk.

Chapter 23

RICKETTSIA

yolk sacs
Tissue culture
guinea pigs
seedlings

Characteristic Features

1. *Rickettsia* species are **small obligate intracellular bacilli**.
2. They have a Gram-negative cell wall structure. *Rickettsiae* are best stained by Giemsa stain.
3. They cannot grow on artificial laboratory media, but can be isolated by inoculation of guinea pigs, mice, yolk sacs of chicken embryos or cell cultures.
4. Organisms are maintained in nature by arthropods transmission.
5. *Rickettsia* contains several species (Table 13).

Table (13): Common *Rickettsia* species

Species	Disease	Typical mode of transmission	Natural cycle
Typhus group: <i>R. prowazekii</i>	① Louse-borne typhus or Epidemic typhus (Worldwide)	Infected louse faeces rubbed into broken skin, mucous membranes or inhaled as aerosol	Human-louse cycle
<i>R. prowazekii</i>	② Brill-Zinsser disease (Worldwide)	Recurrence years after primary attack of louse-borne typhus due to previous latency.	
<i>R. typhi</i>	③ Murine (endemic) typhus (Worldwide)	Infected flea faeces rubbed into broken skin, mucosa or inhaled as aerosol	Rat-flea cycle
Spotted fever group: <i>R. rickettsii</i>	Rocky Mountain spotted fever (Western hemisphere)	Tick bite	Trans-ovarian in ticks
<i>R. akari</i>	Rickettsialpox (USA)	Mite bite	Trans-ovarian in mites
<i>R. conorii</i>	Boutonneuse fever (Africa, Middle East, Southern Europe)	Tick bite	Trans-ovarian in ticks
<i>Orientia</i> * <i>tsutsugamushi</i>	Scrub typhus (Japan, Eastern Asia, Australia)	Chigger (larval mite) bite	Trans-ovarian in mites

plague
By fleas
&
rats

* *Orientia* is now classified as a separate genus.

Pathogenesis and clinical significance

Rickettsiae infect the endothelial cells lining the blood vessels, leading to increased vascular permeability and focal haemorrhages. *Rickettsial* disease is usually accompanied by fever, headache and skin rash. Rocky Mountain spotted fever, louse-borne typhus and scrub typhus are life-threatening illnesses even for young healthy people. In severe cases, rickettsial encephalitis with coma, convulsions and pulmonary oedema are grave conditions that are often fatal.



Laboratory diagnosis

Unfortunately, serologic tests cannot give diagnosis of the acute phase of the disease. Early diagnosis is only possible by **direct detection and isolation** by inoculation of guinea pigs, mice, yolk sacs of chicken embryos or cell cultures.

Antimicrobial susceptibility

Tetracycline is the drug of choice and **chloramphenicol** is an alternative.

Table (14): Comparison between the very small filterable ($0.45 \mu\text{m}$) bacteria (*Mycoplasma*, *Chlamydia*, *Coxiella* and *Rickettsiae*) and the viruses:

Property	<i>Mycoplasma</i>	<i>Chlamydiae</i>	<i>Coxiella</i>	<i>Rickettsiae</i>	Virus
Has both RNA and DNA	Yes	Yes	Yes	Yes	No
Synthesizes proteins with own enzymes	Yes	Yes	Yes	Yes	No
Replicates without host nucleic acid	Yes	Yes	Yes	Yes	No
Division by binary fission	Yes	Yes	Yes	Yes	No
Inhibited by antibiotics that stop protein synthesis	Yes	Yes	Yes	Yes	No
Grows on cell free Media	Yes	No	No	No	No
Requires sterols	Yes	No	No	No	No
Generates Metabolic Energy	Yes	No	Yes	Yes; but ATP parasite	No
Arthropod-borne transmission	No	No	Insignificant	Vital	ARBO viruses

G.R. they are not virus
because s.d.g!
points

Chapter 24

ACTINOMYCETES

@G.R. it was thought to be fungus?
@G.R. it is not fungus?

Ans

① Actinomycetes are a group of organisms which grow as branching filaments (like fungi). However, they are true bacteria as evidenced by:

1. Presence of a cell wall like Gram positive bacteria.
2. Lack of nuclear membrane and mitochondria.
3. Multiplication by simple binary fission.
4. Susceptibility to antibacterial but not to antifungal chemotherapeutic agents.



The genera of actinomycetes of medical importance include:

A. **Aerobic** actinomycetes that are:

- Gram-positive, partially acid-fast bacilli: **Nocardia**.
- Gram-positive, non acid-fast bacilli: **Actinomadura** and **Streptomyces** (streptomyces produce about 85% of all antibiotics known to man).

No card.
Aerobic → Actinomadura
An aerobic → Streptomyces
An aerobic → Actinomyces

B. **Anaerobic** actinomycetes: Gram-positive, non acid-fast bacilli: **Actinomyces**.

They cause three major types of infections:

1. Actinomycosis.
2. Nocardiosis.
3. Mycetoma (actinomycetoma or actinomycotic mycetoma) see chapter 25.

@ Cervicofacial
@ endometritis

Actinomycosis

→ Molar tooth

→ Actinomyces israelii
→ endogenous transm.

It is a chronic suppurative disease commonly caused by **Actinomyces israelii**.

The organism is present as a normal flora of the mouth and vagina.

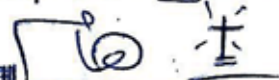
Pathogenesis: Infection is **endogenous** following local trauma where the organism invades the tissues. The disease is characterized by persistent swelling, supuration and formation of abscesses with draining sinuses. The pus contains yellow granules (sulphur granules). The most common clinical forms are:

① **Cervicofacial infection** where the face, neck and mandible are affected. It is usually related to poor dental hygiene and may be initiated by tooth extraction or some other trauma to the mouth or jaw.

② **Chronic endometritis** associated with the use of intrauterine contraceptive devices.

ع اللوب

Sulphur granules



Laboratory diagnosis: The sulphur granules are crushed and subjected to:

1. Microscopic examination which reveals filamentous branching Gram-positive bacilli and coccoid forms.
2. Culture under anaerobic conditions for at least 2 weeks. The colonies are identified by morphology and immunofluorescence staining. Colonies have "molar tooth" appearance on agar.

Treatment

1. Surgical drainage of pus.
2. Administration of antibiotics as penicillin, clindamycin and erythromycin for 4-6 weeks or longer.

Nocardiosis

A. Nocardiosis caused by *Nocardia asteroides* includes:

- 1- **Pulmonary nocardiosis:** A bronchopneumonia that can be easily confused with tuberculosis or pulmonary malignancy.
- 2- **Brain abscess:** a combination of current or recent pneumonia and focal CNS signs suggests *Nocardia* infection.
- 3- **Cutaneous nocardiosis:** pustules, fever and tender regional lymphadenitis.

B. *Nocardia brasiliensis* may be involved as one of the agents causing actinomycetoma (see mycology).

Treatment

1. Trimethoprim- sulfamethoxazole for prolonged period (may be up to 1 year). The *Nocardia* are not susceptible to penicillin.
2. Surgical drainage may be required.

5 Marks
TLO

Chapter 25

MYCOLOGY

Can't synthesis their organic from inorganic
① Fungi
② Not prokaryotic But eukaryotic
③ Heterotrophic reproduce by spore

Characteristics of Fungi

- They are eukaryotic organisms.
- They reproduce by means of spores. Both sexual (meiotic) and asexual (mitotic) spores may be produced.
→ By Budding
- Vegetative forms may be unicellular (yeasts) or composed of hyphae.
- Cell walls are composed mostly of chitin.
- Fungal cell membrane contains ergosterol
- They are heterotrophic, not autotrophic.

MCQ
all following except

Compare bet. filament & yeast fungi

Classification of fungi

A. Morphological classification: 3 types:

1- **Filamentous fungi (moulds)**: These grow as hyphae (septate or non-septate). On artificial media, they form large filamentous colonies. They reproduce by the production of various kinds of spores (conidia). Examples include *Aspergillus*, *Penicillium* and the dermatophytes (*Trichophyton*, *Microsporum* and *Epidermophyton*).

Conidia = spores
(just as Candida)

2- **Yeasts**: These grow as single cells. On artificial media, they form compact colonies. They reproduce by budding and may form pseudohyphae. Examples include *Cryptococcus neoformans*, *Malassezia* and *Candida* species.

3- **Dimorphic**: i.e. have 2 forms of growth:

- At 22°C on artificial media, they grow as hyphae.
 - At 37°C on enriched media or in tissue, they grow as yeasts.
- Examples include *Histoplasma capsulatum* and *Coccidioides immitis*.

give account on dimorphic
it's 2 forms
① filamentous 22°C artificial
② yeast 37°C enriched

B. Clinical classification: According to its relation to disease:

1- **Superficial mycoses**: Affecting the horny layer of the skin, e.g. *Pityriasis versicolor*.

by Malassezia furfur

2- **Cutaneous mycoses**: Affecting the deep layers of the skin, e.g. candida and dermatophytes.

3- **Subcutaneous mycoses**: In which fungi present in the soil are implanted in the subcutaneous tissue by trauma, e.g. mycetoma.

4- **Deep (systemic) mycoses**: These are usually caused by fungi that live free in nature, i.e. opportunistic. Most infections are subclinical. However, some cases may develop severe and even fatal infection, e.g. pneumocystis pneumonia, blastomycosis, cryptococcosis, histoplasmosis and, occasionally, candidiasis.

5- **Mycotoxicosis**: It is produced by consumption of food containing fungal toxins, e.g.:

- **Mushroom poisoning** which causes damage to liver, kidney and bone marrow.
- **Aflatoxin of *Aspergillus flavus*** may cause chronic liver damage and cancer.

6- **Allergic disorders**: Spores of free living fungi as ***Aspergillus*** may be the allergen in some cases of atopy (asthma, hay fever, urticaria, etc.).

Diagnosis of fungal infections

A. **Specimens** are collected according to the site of infection such as **skin scales**, nail clippings, hairs, **respiratory secretions**, **biopsies**, **blood**...etc.

B. Direct detection

1- **Microscopical examination of unstained preparations** to demonstrate hyphae, spores or yeast cells. In case of superficial and cutaneous mycoses, the specimen **skin scales, nail clippings or hairs** is first mounted with 10-20% KOH to dissolve keratin.

2- **Microscopical examination of stained preparations**: Different stains are used, e.g. **Gram's stain**, **India ink**, **Periodic Acid Schiff (PAS)**, silver stains and **lactophenol cotton blue stain**.

3- **Antigen detection** in the specimen (e.g. **cryptococcosis** and **aspergillosis**).

C. **Cultivation**: Different culture media are recommended. The most commonly used is **Sabouraud's dextrose agar (SDA)**. Growth of most fungi is better at 25-30°C. If deep mycotic infection is suspected (which is usually caused by dimorphic fungi), enriched media are inoculated and incubated at 37°C to allow growth of the yeasty phase of the fungus.

D. Identification of culture growth by:

- 1- Colony morphology including the **surface** and **reverse** views of the growth is the principal way for identifying fungi.
- 2- Microscopic examination of **wet** and **stained smears** to distinguish the different types of **hyphae** and **conidia (spores)**.
- 3- Biochemical tests.

E. **Serodiagnosis**: Detection of specific antibody may help in the diagnosis of systemic mycoses.

F. **Skin testing (delayed type hypersensitivity)**.

(X) **Antifungal Drugs**: Because fungi are eukaryotes, the range of non-toxic systemically active antifungal drugs is still limited. The most commonly used drugs are amphotericin B, griseofulvin, flucytosine, terbinafine, fluconazole, ketoconazole, itraconazole and mycostatin (nystatin).

McQ

±

Pityriasis versicolor

by *Malassezia furfur*

It is a common fungus infection of the horny layer of the skin. It affects the upper part of the trunk and sides of the neck. It appears as scaly macules, either hyperpigmented or hypopigmented. The causative organism is *Malassezia furfur*.

Dermatophytes

Ring worm = tinea like
bec. spread radially and heal in centre

Three Genera of dermatophytes infect man. These are: *Microsporum*, *Trichophyton* and *Epidermophyton*. They affect the keratinized tissue (hair, nail and skin). Infection is transmitted by direct or indirect contact. The disease is characterized by being superficial, extends radially and heals at the centre to form circular lesions called ringworm. The sources of infection may be man (anthropophilic), animals as dogs and cats (zoophilic) and soil (geophilic).

Clinical types: Ringworm is usually referred to as tinea. According to the site, there are several types of ringworm infections:

- ① Tinea capitis (ringworm of the scalp).
- ② Tinea pedis (athlete's foot).
3. Tinea unguinum (ringworm of nails).
4. Tinea circinata (ringworm of non-hairy skin).
5. Tinea cruris (ringworm of the skin of the groin).
6. Tinea barbae (ringworm of the skin of beard).

invasive
non invasive
Allergy

Aspergillosis

Mycotoxicosis by aflatoxin

It is caused by *Aspergillus fumigatus*, rarely by *Aspergillus niger* and *A. flavus*. The spores are found in air and are continuously inhaled.

Clinical presentations

1. Allergy, e.g. bronchial asthma (type I hypersensitivity).
2. Non-invasive infections, e.g. colonization of a preexisting lung cavity and otitis.
3. Invasive infection occurs in severely immunocompromised patients with leukaemia, organ transplant recipients and patients under steroid therapy.
4. Mycotoxicosis: Aflatoxin of *A. flavus* is hepatotoxic and carcinogenic.

or ✓

Pneumocystis

by *Pneumocystis carinii*
→ *Jeroveci*

Pneumocystis carinii is classified as a yeast on the basis of molecular analysis, but medically many still think of it as a protozoan or as an unclassified organism. Most people are infected with *P. carinii* by the age of four and develop no symptoms, unless they are immunocompromised. It causes **interstitial pneumonia** in association with HIV infection, primary immunodeficiency, prolonged steroid treatment, organ transplantation and cancers. The organism can be detected in clinical specimens by PCR or after staining with silver stain.

In 2002, taxonomists renamed the human species of *Pneumocystis* as *P. jiroveci* and recommended that *P. carinii* be used only to describe the rat species of *Pneumocystis*.



Candidiasis (moniliasis) is most frequently caused by *Candida albicans* and rarely due to infection by other species, e.g. *C. stellatoidea*, *C. krusei*...etc. *Candida albicans* is present as normal flora in the oral cavity, vagina and intestine.

Predisposing factors to candidiasis include:

1. Diabetes.
2. Broad-spectrum antibiotic treatment (superinfection).
3. Steroid therapy.
4. Immunosuppression.
5. Prolonged exposure to water pregnancy and old age.

Clinical features of candidiasis

Cutaneous: Affecting skin and mucous membranes: (superficial)

- Oral thrush.
- Vulvovaginitis.
- Paronychia (nail infection).
- Interdigital.
- Intertrigo (between skin folds), e.g. diaper rash.

Chronic mucocutaneous candidiasis is usually associated with T-cell deficiency.

Systemic: Affecting the lung or kidney. It usually complicates an underlying disease as TB, malignancy or immunosuppression.

Laboratory diagnosis

1. Specimens from skin, mouth, vagina, sputum...etc.
2. Microscopic examination of a Gram-stained smear for the presence of Gram positive oval, budding yeast cells and pseudohyphae.
3. Cultivation: *C. albicans* can grow on most culture media at 37°C. The selective medium is Sabouraud's dextrose agar (SDA) containing chloramphenicol. After 1-2 days, yeast colonies appear creamy white, pasty with yeasty odour. *C. albicans* is differentiated from other *Candida* species by the followings:

• Formation of germ tubes in serum at 37°C within 1-2 hours.

• Formation of chlamydospores on corn meal agar.

• Biochemical reactions.

differentiate from other *Candida*

Diagnosis = Large gelatinous caps.
mucoid colonies
urease +ve



Cryptococcosis

excreta of Bird
by *Cryptococcus neoformans*
meningitis & pneumonia

It is caused by *Cryptococcus neoformans*. The organism is present in the soil contaminated with excreta of birds particularly pigeons. Infection occurs by inhalation. It may result in lung infection (cryptococcus pneumonia). Spread to the central nervous system leads to **meningitis**. Reduced cell-mediated immunity, especially in AIDS patients, predisposes to severe disease.

Laboratory diagnosis

1. Detection of the organism in sputum or CSF. A smear stained with India ink will demonstrate *Cryptococcus neoformans* as budding yeast cells surrounded by a large gelatinous capsule.
2. Culture: on SDA, the organism gives mucoid colonies. Growth is identified by characteristic morphology, urease positive and pathogenicity to mice.
3. Detection of capsular antigen in the CSF, by latex agglutination test.
4. Detection of specific antibodies in the patient's serum.

Mycetoma (Madura Foot or Maduromycosis)

Mycetoma is a clinical syndrome characterized by granuloma, tumefaction, multiple abscesses, draining sinuses and pus with granules. It is a localized infection that involves cutaneous and subcutaneous tissue, fascia and bone. It usually affects the foot and rarely the hands and buttocks. The organisms involved are present in the soil and are implanted, by trauma, into the tissues especially in bare footed people. Therefore, lesions are localized at the site of the trauma. According to causative organisms there are two types of mycetoma (Table 15):

1. True fungi (Eumycotic mycetoma).
2. Actinomycetes (Actinomycotic mycetoma).

It is important to know whether the mycetoma is caused by fungi or actinomycetes because actinomycotic mycetoma will respond to antibiotics while fungal infection will not. The latter needs surgical treatment up to amputation.

Table (15): Eumycotic (fungal) and actinomycotic mycetoma

	Actinomycotic mycetoma	Eumycotic mycetoma
Causative agent	Bacterial: e.g. <i>Actinomyces madurae</i> , <i>Nocardia brasiliensis</i> and <i>Streptomyces somaliensis</i>	Fungal: e.g. <i>Madurella mycetomatis</i> , <i>Madurella grisea</i> and <i>Allescheria boydii</i>
Colour of granules	Mainly yellow (sulphur)	Mainly black and white
Microscopic exam	Thin fragmented filaments	Thick hyphae and spores
Culture	On blood agar, aerobic and anaerobic	On SDA, incubated at 30°C → Sabouraud dextrose agar
Chemotherapy	Effective	- No or poor response - Needs surgical treatment and may be amputation

What importance to differ bet. Fungus & Bact?
Ans - bec differ to treat
Fungus → Surgical
Bact → Antibiotic

Practical Sessions Attendance Sheet

First Term



Student's Name:

Student's Number:

Section:

Session	Subject	Date	Signature	Remarks
1	Methods of identification Microscopy & Bacterial Morphology			
2	Staining methods (Gram & Z- N stains)			
3	Culture media & methods of anaerobic cultivation			
4	Biochemical reactions			
5	Other tests for identification: Animal pathogenicity, phage typing & molecular methods			
6	Immunological methods "I"			
7	Immunological methods "II"			
8	Sterilization & disinfection			
9	Antibiotic Sensitivity tests			

It is more blessed to give than to receive.